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## *ORIGINAL ARTICLES*

### TEMPORARY DISTURBANCES DUE TO LOCAL ANESTHETICS*


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TEMPORARY disturbances to the system, usually slight, but sometimes of a severe character, may be expected following the use of local anesthetics. These disturbances, chiefly in the circulatory system, are of considerable concern to both physicians and dentists.

The drugs used locally are cocain and adrenalin or novocain and adrenalin, often accompanied by morphine and atropine. The action of each of these drugs when injected into the circulation has been thoroughly worked out by many investigators. The action of two of the drugs used in succession, either applied with a sponge upon or injected under the mucous membrane, has not been so fully investigated. Adrenalin or pituitrin, for instance, do not have the same effect given by way of the stomach that they have when administered hypodermically. It was therefore considered best for experimental purposes to administer the drugs exactly as they are used in medical practice, so that the results might be comparable.

The object of this work, then, was to determine changes taking place in the respiratory and circulatory systems when the commonly used local anesthetics were applied to animals by the same method used in medical practice.

#### EXPERIMENTAL WORK

Dogs were used as subjects. The work was done on the animals under general anesthesia induced by the intraperitoneal injection of 1. c.c. of 25 per cent of chloretone in almond oil, per kilogram of body weight. Kymographic records were taken of arterial pressure from the carotid and the intracranial venous pressure from the Torcular Herophili. The respiratory


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TABLE I

EFFECTS OF SUBMUCOUS INJECTIONS OF ADRENALIN AND ADRENALIN-COCAIN

| DOG | ARTERIAL PRESSURE | | | VENOUS PRESSURE | | | HEART RATE | | | HEART AMPLITUDE | | | RESPIRATION RATE | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BEFORE | AFTER ADREN. | AFTER ADREN.-COCAIN | BEFORE | AFTER ADREN.- | AFTER ADREN.-COCAIN | BEFORE | AFTER ADREN. | AFTER ADREN.-COCAIN | BEFORE | AFTER ADREN. | AFTER ADREN.-COCAIN | BEFORE | AFTER ADREN. | AFTER ADREN.-COCAIN |
| 1 | 52 | 76 | 170 | .5 | .8 | – | 174 | 174 | 156 | 8 | 8 | 11 | – | – | – |
| 2 | 42 | 42 | 150 | .2 | .15 | 1.1 | 144 | 150 | 96 | 7 | 5 | 20 | – | – | – |
| 3 | 70 | 72 | 190 | 1.9 | 2.0 | 6.2 | 240 | 222 | 120 | 5 | 5 | 25 | 21 | 24 | 48 |
| 4 | 70 | 70 | 230 | .9 | .9 | 4.2 | 162 | 162 | 144 | 6 | 6 | 12 | 54 | 66 | 72 |
| 5 | 66 | 66 | 230 | .9 | .9 | 8.9 | 126 | 126 | 126 | 13 | 13 | 45 | 21 | 21 | 72 |
| Average | 60 | 65 | 194 | .9 | .9 | 5.1 | 169 | 167 | 128 | 8 | 7 | 23 | 32 | 37 | 64 |

TABLE II

EFFECTS OF SUBMUCOUS INJECTIONS OF ADRENALIN AND ADRENALIN-COCAIN AFTER MORPHINE-ATROPINE

| DOG | ARTERIAL PRESSURE | | | VENOUS PRESSURE | | | HEART RATE | | | HEART AMPLITUDE | | | RESPIRATION RATE | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | BEFORE | AFTER ADREN. | AFTER ADREN.-COCAIN | BEFORE | AFTER ADREN. | AFTER ADREN.-COCAIN | BEFORE | AFTER ADREN. | AFTER ADREN.-COCAIN | BEFORE | AFTER ADREN. | AFTER ADREN.-COCAIN | BEFORE | AFTER ADREN. | AFTER ADREN.-COCAIN |
| 1 | 46 | 46 | 184 | 2.5 | 2.5 | 3.2 | 138 | 156 | 144 | 10 | 10 | 12 | 22 | 34 | 22 |
| 2 | 44 | 48 | 180 | .6 | .8 | 4.8 | 126 | 138 | 216 | 10 | 9 | 2 | 24 | 20 | 22 |
| 3 | 78 | 78 | 180 | .6 | .6 | 7.8 | 186 | 180 | 120 | 3 | 2 | 10 | 4 | 20 | 36 |
| 4 | 74 | 64 | 190 | .9 | .8 | 1.8 | 210 | 216 | 180 | 3 | 4 | 3 | 46 | 23 | 36 |
| 5 | 50 | 48 | 140 | 1.1 | 1.1 | 3.2 | 102 | 120 | 54 | 13 | 12 | 20 | 28 | 30 | 10 |
| Average | 58 | 57 | 175 | 1.1 | 1.2 | 4.2 | 152 | 162 | 143 | 8 | 7 | 9 | 25 | 25 | 25 |

rate was obtained from the tracing of arterial pressure. The arterial and venous pressures were measured in millimeters of mercury, and the amplitude of heart beat in millimeters on the tracing. The rate of pulse and respirations per minute were determined.

The venous pressure was taken with the aid of a manometer filled with sodium carbonate solution of a specific gravity just one-tenth that of mercury.

The first group of dogs was given an injection of 33⅓ per cent of the 1:1000 adrenalin, 0.15 c.c. per kilogram of animal. This amount was injected into three or four places in the tonsillar region. After five minutes an injection was made in the same region of a mixture of cocain and adrenalin. This mixture consisted of 33⅓ per cent of adrenalin (1:1000) and 13⅓ per cent cocain. The arterial pressure, venous pressure, rate and amplitude of heart beat and respiratory rate were estimated for the five animals. The results are given in Table I.

The second group of animals was treated in the same way except that half an hour before the adrenalin was injected the animals were given subcutaneously ⅛ c.c. of a 4 per cent morphine sulphate and 1/10 c.c. of 1/10 per cent atropine sulphate per kilogram of body weight. The results are given in Table II.

TABLE III

EFFECTS OF SUBMUCOUS INJECTIONS OF ADRENALIN AND COCAIN

| DOG | ARTERIAL PRESSURE | | VENOUS PRESSURE | | HEART RATE | | HEART AMPLITUDE | | RESPIRATION RATE | |
|---|---|---|---|---|---|---|---|---|---|---|
| | BEFORE COCAIN | AFTER COCAIN | BEFORE COCAIN | AFTER COCAIN | BEFORE COCAIN | AFTER COCAIN | BEFORE COCAIN | AFTER COCAIN | BEFORE COCAIN | AFTER COCAIN |
| 1 | 66 | 165 | 0 | 1.1 | 198 | 180 | 8 | 4 | 64 | 54 |
| 2 | 80 | 110 | 1.1 | 1.2 | 174 | 182 | 3 | 3 | 26 | 42 |
| 3 | 84 | 208 | 1.0 | 2.2 | 138 | 162 | 5 | 6 | 24 | 50 |
| 4 | 86 | 150 | 2.6 | 4.8 | 132 | 144 | 9 | 6 | 56 | 52 |
| 5 | 94 | 116 | 1.8 | 3.7 | 144 | 144 | 5 | 6 | 56 | 48 |
| Average | 82 | 150 | 1.3 | 2.6 | 157 | 162 | 6 | 5 | 45 | 49 |

The third group was given the same dose of adrenalin in the usual manner and five minutes later, 0.15 c.c. per kilogram of weight, a solution containing 13⅓ per cent cocain was injected into the tonsillar region. The results are given in Table III. The observations were made just before the cocain was injected and at the height of its action.

The fourth, fifth and sixth groups show the effect that swabbing the throat with cocain had on the reaction to the injections of adrenalin.

The fourth group of animals was given 0.15 c.c. of 50 per cent adrenalin (1:1000) per kilogram of weight. After all effects had passed off the throat was swabbed vigorously with 20 per cent cocain. Within 2 minutes the same dose of adrenalin as that given previously was injected into the tonsillar region. The results are given in Table IV.

The animals of the fifth group were given a mixture containing 50 per cent adrenalin (1:1000) and 20 per cent novocain. This was injected into the tonsillar region—0.15 c.c. per kilogram of the animal's weight. After the

TABLE IV

EFFECTS OF SUBMUCOUS INJECTIONS OF ADRENALIN PRECEDED BY SWABBING THROAT WITH COCAIN

| DOG | ARTERIAL PRESSURE | | | VENOUS PRESSURE | | | HEART RATE | | | HEART AMPLITUDE | | | RESPIRATION RATE | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NORMAL | ADREN-ALIN | ADREN. AFTER COCAIN | NORMAL | ADREN-ALIN | ADREN. AFTER COCAIN | NORMAL | ADREN-ALIN | ADREN. AFTER COCAIN | NORMAL | ADREN-ALIN | ADREN. AFTER COCAIN | NORMAL | ADREN-ALIN | ADREN. AFTER COCAIN |
| 1 | 94 | 84 | 234 | 2.0 | 1.2 | 3.8 | 138 | 126 | 102 | 6 | 6 | 12 | 34 | 30 | 18 |
| 2 | 70 | 90 | 230 | .9 | 1.0 | 5.2 | 150 | 150 | 162 | 10 | 9 | 7 | 10 | 36 | 41 |
| 3 | 72 | 78 | 160 | 1.6 | 1.7 | 4.6 | 210 | 186 | 204 | 7 | 7 | 10 | 50 | 50 | 100 |
| 4 | 100 | 80 | 190 | – | – | – | 150 | 180 | 168 | 7 | 7 | 14 | 10 | 24 | 40 |
| 5 | 80 | 82 | 170 | 1.6 | 1.8 | 4.2 | 168 | 174 | 180 | 4 | 4 | 15 | 30 | 48 | 24 |
| Average | 83 | 83 | 197 | 1.5 | 1.4 | 4.4 | 163 | 163 | 163 | 7 | 7 | 12 | 27 | 38 | 45 |

TABLE V

EFFECTS OF SUBMUCOUS INJECTIONS OF NOVOCAIN AND ADRENALIN PRECEDED BY SWABBING THROAT WITH COCAIN

| DOG | ARTERIAL PRESSURE | | | VENOUS PRESSURE | | | HEART RATE | | | HEART AMPLITUDE | | | RESPIRATION RATE | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | NORMAL | NOVO-ADREN. | NOVO-ADREN. AFTER COCAIN | NORMAL | NOVO-ADREN. | NOVO-ADREN. AFTER COCAIN | NORMAL | NOVO-ADREN. | NOVO-ADREN. AFTER COCAIN | NORMAL | NOVO-ADREN. | NOVO-ADREN. AFTER COCAIN | NORMAL | NOVO-ADREN. | NOVO-ADREN. AFTER COCAIN |
| 1 | 78 | 118 | 188 | 1.1 | 1.3 | 2.6 | 156 | 180 | 192 | 5 | 4 | 4 | 36 | 36 | 76 |
| 2 | 50 | 116 | 140 | 1.1 | 1.6 | 2.1 | 180 | 144 | 120 | 5 | 6 | 7 | 50 | 34 | 42 |
| 3 | 46 | 36 | 172 | 2.2 | 2.0 | 9.2 | 138 | 138 | 180 | 8 | 6 | 2 | 44 | 58 | 32 |
| 4 | 70 | 94 | 138 | .6 | .9 | 1.5 | 150 | 210 | 210 | 8 | 7 | 2 | 40 | 18 | 24 |
| 5 | 112 | 100 | 190 | 1.8 | 1.6 | 2.5 | 150 | 168 | 120 | 3 | 3 | 12 | 22 | 12 | 62 |
| Average | 71 | 93 | 166 | 1.4 | 1.5 | 3.6 | 155 | 168 | 164 | 6 | 5 | 5 | 38 | 32 | 47 |

action of this injection had disappeared the throat was swabbed with 20 per cent cocain. Within two minutes the mixture of novocain and adrenalin was again injected. The results are given in Table V.

The sixth group was given 0.15 c.c. of 50 per cent of adrenalin (1:1000) per kilogram of weight. When the effect of the adrenalin's action had disappeared the throat was swabbed with 20 per cent cocain. Observations were made just before the cocain was applied and at the height of its action. The results are arranged in Table VI.

## DISCUSSION

The first problem was the determination of the proper submucous dosage of adrenalin for comparative purposes. This included not only the amount of the adrenalin, but the concentration of the preparation. After much time had been consumed experimenting on a considerable number of animals the dosage of 0.15 c.c. of 33⅓ per cent adrenalin (1:1000) per kilogram of the animal's weight was decided upon. This dose was active to the minimum and thus allowed a maximum range of action for any drug used with it.

The submucous dose of cocain used was 0.15 c.c. of 13⅓ per cent solution of cocain hydrochloride per kilogram of body weight.

The first experiment was designated to demonstrate whether any considerable reaction takes place when minimal doses of adrenalin and cocain are injected together. Adrenalin alone changed the average arterial pressure from 60 to 65 mm., venous pressure from 0.9 to 0.9 mm., rate of heart beat from 169 to 167 beats per minute, force of heart beat from 8 to 7 and respiratory rate from 32 to 37 per minute. Expressed in per cent, adrenalin produced an increase in arterial pressure of 8, in respiratory rate of 16 and a decrease in rate of heart beat of 1, and in amplitude of heart beat of 13. Venous pressure was not changed by this dosage of adrenalin. In general it may be stated that the effect of this dose of adrenalin is minimal.

This same series of animals was given submucously per kilogram of weight 0.15 c.c. of a solution containing adrenalin 33⅓ per cent and cocain hydrochloride 13⅓ per cent. This solution was given after the reaction of the adrenalin alone had completely disappeared. The maximum action of this mixture produced an average of 194 mm. of arterial pressure, 5.1 mm. of venous pressure, a rate of heart beat of 128 per minute, a heart amplitude of 23 mm. and a respiratory rate of 54 per minute. Expressed in percentage of the normal there was a great increase in all but one of the factors measured. The rate of heart beat was decreased 18 per cent. There was an increase of 233 per cent in arterial pressure, 467 per cent in venous pressure, 187 per cent in amplitude of heart beat and 100 per cent in respiratory rate. In general, it may be stated that the reaction was enormous except in the rate of the heart beat.

It is very significant that the increase of arterial pressure was only about half of the increase of the intracranial venous pressure. This condition would produce cerebral asphyxia, which might induce all of the temporary disturbances occurring in the use of local anesthetics in medical practice.

Cerebral asphyxia causes a feeling of smothering, accompanied by some excitement and followed by faintness and dimness of vision.

It is a common practice to give hypodermic injections of morphine and atropine a short period before an operation which is to be done under local anesthesia. It was a question whether such premedication would increase or decrease the disturbances produced by cocain and adrenalin. The principal influence that morphine might exert on the factors under consideration is a retardation of the heart by vagus stimulation and also a retardation of

TABLE VI

EFFECTS OF SWABBING THROAT WITH COCAIN

| | ARTERIAL PRESSURE | | VENOUS PRESSURE | | HEART RATE | | HEART AMPLITUDE | | RESPIRATION RATE | |
|---|---|---|---|---|---|---|---|---|---|---|
| DOG | BEFORE | AFTER | BEFORE | AFTER | BEFORE | AFTER | BEFORE | AFTER | BEFORE | AFTER |
| 1 | 164 | 210 | 2.5 | 5.2 | 168 | 156 | 6 | 13 | 24 | 44 |
| 2 | 130 | 130 | .9 | 1.0 | 138 | 144 | 5 | 8 | 66 | 60 |
| 3 | 88 | 94 | .8 | .7 | 114 | 138 | 9 | 7 | 34 | 64 |
| 4 | 88 | 86 | .5 | .5 | 144 | 150 | 9 | 8 | 52 | 48 |
| 5 | 108 | 152 | 1.0 | 1.3 | 144 | 144 | 12 | 7 | 26 | 38 |
| Average | 116 | 134 | 1.1 | 1.7 | 142 | 146 | 8 | 9 | 40 | 51 |

TABLE VII

TABLE OF AVERAGES

| EXPERIMENT | | ARTERIAL PRESSURE | VENOUS PRESSURE | HEART RATE | HEART AMPLITUDE | RESPIRATION RATE |
|---|---|---|---|---|---|---|
| 1 | Normal | 60 | .9 | 169 | 8 | 32 |
| 1 | Adrenalin After | 65 | .9 | 167 | 7 | 37 |
| 1 | Adrenalin and Cocain After | 194 | 5.1 | 128 | 23 | 64 |
| 2 | Morphine-Atropine K ½ Hour Preceding | 58 | 1.1 | 152 | 8 | 25 |
| 2 | Adrenalin After | 57 | 1.2 | 162 | 7 | 25 |
| 2 | Adrenalin and Cocain After | 175 | 4.2 | 143 | 9 | 25 |
| 3 | Cocain—Long After Adren. | 82 | 1.3 | 157 | 6 | 45 |
| 3 | Cocain After | 150 | 2.6 | 162 | 5 | 49 |
| 4 | Normal | 83 | 1.5 | 163 | 7 | 27 |
| 4 | Adrenalin After | 83 | 1.4 | 163 | 7 | 38 |
| 4 | Adrenalin After Cocain Swab After | 197 | 4.4 | 163 | 12 | 45 |
| 5 | Normal | 71 | 1.4 | 155 | 6 | 38 |
| 5 | Adrenalin—Novocain After | 93 | 1.5 | 168 | 5 | 32 |
| 5 | Adren.—Novocain After Cocain S. After | 166 | 3.6 | 164 | 5 | 47 |
| 6 | Before Cocain Swab—After Adrenalin | 116 | 1.1 | 142 | 8 | 40 |
| 6 | After Cocain Swab | 134 | 1.7 | 146 | 9 | 51 |

the respiration by depression of the respiratory center. Atropine would exert its chief influence to increase the rate of heart beat through paralysis of the vagus nerve endings. This action of atropine would tend to counterbalance the action of morphine on the rate of the heart beat.

These conclusions reached through deduction could only be proved or disproved by making the actual tests. Consequently a group of animals was given hypodermic injections of morphine and atropine sulphate in doses comparable with those used in medical practice. The injections were made

half an hour before the submucous injections were begun. The same drugs in the same doses were used as with the preceding set of animals.

The increases of 202 and 282 per cent in the arterial and venous pressures are not as great as those noticed in animals without the morphine and atropine. The decrease of the rate of heart beat was only 6 per cent, while without premedication it was 18 per cent. The most striking difference was in the absence of any change in either the amplitude of heart beat or respiratory rate. This constancy was probably due to the action of atropine. The effect of the morphine, depressing the respiratory center, seems to have been just sufficient to counterbalance the stimulation through asphyxia of the respiratory center produced by the adrenalin and cocain. A certain amount of the reduction of the reactions to cocain and adrenalin observed in the premedication with morphine and atropine must be due to the increased depth of general anesthesia produced by the chloretone and morphine.

In view of these various phases of action it must be concluded that premedication with morphine and atropine does not reduce the main factors involved in the temporary disturbances from injections of cocain and adrenalin. It may be significant that from the considerable number of animals that had been given the cocain only two died and these two had both been treated with morphine and atropine.

A third group of animals was used in the attempt to explain the cause of the great changes in blood pressure, action of the heart and respiration produced by injections of adrenalin and cocain. Adrenalin administered alone in the quantity and manner employed was not the cause of the changes, as is shown by the results obtained from the first group of dogs. The third group was used to estimate the effect of cocain alone. Adrenalin was given before the cocain in order that the comparison might be made with the first group. The cocain increased arterial pressure 83 per cent, venous pressure 100 per cent, rate of the heart beat 3 per cent and rate of respiration 9 per cent but decreased the amplitude of heart beat 13 per cent. It is very clear that the blood pressure was altered two to four times as much by the combined action of adrenalin and cocain as by the individual action of either. This synergistic action of cocain and adrenalin is well known to occur when adrenalin is injected intravenously after intravenous injection of cocain. That marked synergy is present when adrenalin and cocain are injected submucously has not been demonstrated.

Since cocain is capable of so great an influence over the reaction to adrenalin, it was thought interesting to determine whether such small amounts of cocain as are absorbed when the pharynx is swabbed with 20 per cent cocain were sufficient to increase the sensitiveness to adrenalin.

A submucous injection of 0.15 c.c. of a 50 per cent adrenalin (1:1000) was used as the dosage. After this had been given time to disappear 20 per cent cocain was swabbed well on the pharynx and shortly after another similar dose of adrenalin was injected. The first injections of adrenalin produced no change in any of the factors except a decrease of 7 per cent in venous pressure and an increase of 41 per cent in rate of respiration. The

second injection of adrenalin which followed the swabbing of cocain produced an increase of 13 per cent in arterial pressure, 193 per cent in venous pressure, 71 per cent in amplitude of heart beat, and 67 per cent in rate of respiration. No change was produced in rate of heart beat.

Novocain is one of the most commonly used local anesthetics. That novocain with adrenalin does not eliminate the strong synergistic action of cocain on the reaction to adrenalin is shown by the results obtained with the fifth group of animals.

The preceding method was followed except that the solution of adrenalin contained also 20 per cent of novocain. The first injection of novocain and adrenalin produced an increase of 31 per cent in arterial pressure, 7 per cent

TABLE VIII

TABLE OF AVERAGES—EXPRESSED IN PER CENT OF THE NORMAL

| EXPERIMENT | | ARTERIAL PRESSURE | VENOUS PRESSURE | HEART RATE | HEART AMPLITUDE | RESPIRATION RATE |
|---|---|---|---|---|---|---|
| 1 | Normal | 100 | 100 | 100 | 100 | 100 |
| 1 | Adrenalin After | 108 | 100 | 99 | 87 | 116 |
| 1 | Adrenalin and Cocain After | 323 | 567 | 82 | 287 | 200 |
| 2 | Morphine and Atropine (½ hr. preceding) | 100 | 100 | 100 | 100 | 100 |
| 2 | Adrenalin After | 98 | 109 | 107 | 87 | 100 |
| 2 | Adrenalin and Cocain After | 302 | 382 | 94 | 112 | 100 |
| 3 | Normal—Long After Adrenalin | 100 | 100 | 100 | 100 | 100 |
| 3 | Cocain After | 183 | 200 | 103 | 83 | 109 |
| 4 | Normal | 100 | 100 | 100 | 100 | 100 |
| 4 | Adrenalin After | 100 | 93 | 100 | 100 | 141 |
| 4 | Adrenalin After Cocain Swab | 237 | 293 | 100 | 100 | 167 |
| 5 | Normal | 100 | 100 | 100 | 100 | 100 |
| 5 | Adrenalin and Novocain | 131 | 107 | 108 | 83 | 84 |
| 5 | Adrenalin and Novocain After Cocain S. | 234 | 257 | 106 | 83 | 124 |
| 6 | Before Cocain Swab—After Adrenalin | 100 | 100 | 100 | 100 | 100 |
| 6 | After Cocain Swab—After Adrenalin | 116 | 155 | 103 | 112 | 127 |

in venous pressure, 8 per cent in rate of heart beat and a decrease of 17 per cent in amplitude of heart beat and 16 per cent in rate of respiration.

The second injection of novocain and adrenalin produced an increase of 134 per cent in arterial pressure, 128 per cent in venous pressure, 6 per cent in rate of heart beat and 24 per cent in respiratory rate. The amplitude of heart beat decreased 17 per cent. Novocain clearly does not interfere with the synergistic action of cocain and adrenalin.

To determine what changes would be accomplished by swabbing 20 per cent cocain on the pharynx a series of animals was taken. These animals were given the same dosage of adrenalin as the fifth group and ten minutes later 20 per cent cocain was swabbed on the pharynx. This swabbing increased the arterial pressure 20 per cent, the venous pressure 55 per cent, the rate of heart beat 3 per cent, amplitude of heart beat 12 per cent and the rate of respiration 12 per cent. Most of these average increases were due to the rather marked reaction of one animal. The other four were very little

affected. These average increases were relatively small compared with the increases due to the swabbing of cocain and the injection of adrenalin.

Therefore we must conclude that swabbing the throat with 20 per cent cocain sensitizes an animal to adrenalin to a marked degree. Lewis and Froelich[1] first demonstrated a synergy between cocain and adrenalin. By intravenous injections of these drugs they showed that adrenalin produced a much greater rise in arterial pressure after the cocain had been administered.

Tatum[2] from a number of observations on the response of the sympathetic nervous system to various stimuli, concludes that cocain increases the responsitivity of the peripheral neuromuscular mechanism of the sympathetic system.

In the Report of the Committee on Local Anesthetics in Ophthalmic Work[3] the following statement is made: "Some observers are inclined to believe that the mild toxic symptoms seen after instillations of solutions of cocain may be due to psychic influences as much as to the toxic effects of the cocain." The results presented seem to bear out the contrary conclusions.

Adrenalin is always used with cocain. If we accept the common view that excitement increases the secretion of adrenalin the reaction may be very much increased by the presence of cocain at the time of the excitement.

## SUMMARY AND CONCLUSIONS

A group of animals was given submucous injections of a minimally active dose of adrenalin, and five minutes later a submucous injection of adrenalin with cocain of the same amount and same concentration. The arterial pressure, venous pressure, heart beat, heart amplitude and respiratory rate were recorded.

A second group of animals was treated in the same way. One hour later a moderate dose of morphine and atropine was given hypodermically.

A third group of animals was given adrenalin in the same manner, and with the same dosage as in Group 1, followed by the usual dose of cocain.

A fourth group of animals was given submucous injections of adrenalin, followed by swabbing the pharynx with 20 per cent of cocain and a repetition of the submucous dose of adrenalin.

A fifth group of animals was given submucous injections of adrenalin and 20 per cent novocain, followed by swabbing of the pharynx with 20 per cent cocain and a repetition of the injection of adrenalin and novocain.

A sixth group of animals was given submucous injections of adrenalin, followed by swabbing of the throat with 20 per cent cocain.

The results of these procedures lead us to the following conclusions:

1. Submucous injections of adrenalin and cocain bring on systemic reactions similar to those produced by intravenous injections of the drug.

2. Submucous injections of cocain and adrenalin are capable of causing enormous increases in arterial and venous pressures. The intracranial venous pressure was increased relatively twice as much as the arterial pressure.

3. Cocain and adrenalin, when injected submucously, act strongly synergistically.

4. Sufficient cocain is absorbed from sponging the pharynx with 20 per cent cocain to increase markedly the sensitiveness of the system to the action of adrenalin.

5. Novocain in a concentation of 20 per cent does not neutralize to any great extent the synergistic action of cocain and adrenalin.

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# RETICULOSIS—INCREASED PERCENTAGE OF RETICULATED ERYTHROCYTES IN THE PERIPHERAL BLOOD*

By Edward B. Krumbhaar, M.D., Ph.D., Philadelphia, Pa.

THE presence of reticulated or "skeined" erythrocytes (hematies granuleuses) in the peripheral blood (as demonstrated by vital staining) has excited interest for many years, and in the last decade has assumed clinical importance as an index of the activity of blood formation. I would suggest as a matter of convenience that when the normal percentage of these cells in the peripheral blood is exceeded, the condition be designated "reticulosis," to replace some such expression as "an increase in the number of reticulated cells," as is now the custom. The word "reticulocyte" might similarly be substituted for "reticulated erythrocytes," but the term "reticulocytosis" (though perhaps more accurate etymologically) seems to me less desirable than the shorter and more convenient term.

In this paper I propose to consider the cytological status of these cells, and offer a few experimental and clinical facts pertaining thereto. Though now always studied by some form of so-called vital staining, they were almost surely first observed by Ehrlich[1] in 1881, in air dried blood smears stained with a saturated aqueous solution of methylene blue. To his credit it should be noted that at that time he considered them as not regressive. In using his method with preparations very freshly spread, I have seen the very fine network that he describes, but it is far less satisfactory than the use of vital staining.

Ehrlich's rather cursory observations were not followed up, although Pappenheim was working on the problem in the nineties.† With the beginning of the new century, however, hematological literature produced many observations and discussions on the nature of the various basophilic substances that are to be found in erythrocytes. The consensus of opinion resulting from these studies (though it must be admitted that but little conclusive proof has been brought forward) is that the reticulation indicates a protoplasmic substance (substantia granulo-filamentosa) and not a nuclear remnant, (being found well developed in nucleated erythrocytes and staining metachromatically with some nuclear stains) and that it indicates a young blood cell and not an old or degenerated cell. The reasons for the latter belief are that reticulocytes are found in great numbers in young infants and rare in adults, also that they are increased in those diseases in which there is an extra demand and an unimpaired source of supply (as in hemolytic

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†I have been unable to find this matter discussed in his inaugural thesis (Berlin 1895), to which references have been made. In a footnote on page 54 of an article in Virchow's Archivk. Pathologische Anatomie, 1899, clix, he speaks of the use of thionin and neutral red as vital stains, but there is no mention of a reticulum in erythrocytes.

jaundice), and diminished or absent where the demand is lessened (as after transfusions) or if the bone marrow function is damaged (as in aplastic anemia). Stainability of the reticulum in many instances parallels polychromatophilic with the Romanowsky method. Enumeration of the polychromatophilic cells, however, cannot replace the reticulocyte count after vital staining, as the sharp distinction in the latter method does not exist in the former; also the former is not so delicate a method, so that very slight reticulations would be lost, and the count therefore always lower. That the two are not necessarily identical is shown by the fact that many polychromatophilic blasts do not contain reticulum, and that in combined staining, the two are not constantly associated.

The basophilic granules or "stippling," such as found in lead poisoning, are usually considered to be of a different nature and probably indicate an abnormal or degenerated cell. The various nuclear remnants (Howell-Jolly bodies, etc.) belong, of course, to a different category. The very widespread distribution of mitochondria in the protoplasm of animal cells, together with certain similarities in behavior, has led to the assumption that the vital staining reticulum was the mitochondria of the young erythrocyte. Until this has been definitely proved, however, it would seem wiser not to confound the two terms, as is occasionally done; furthermore, Key[2] has recently acquired important evidence tending to show that the two substances are not identical. He calls attention to the fact that the reticulum differs from mitochondria, not only in morphology and various staining reactions, but also in its persistence after shedding, its resistance to heat and to solution by water, acetic and other acids, and alcohol, ether and chloroform.

## METHODS

As the reticulum can be demonstrated in the erythrocyte by any method that introduces freshly drawn blood cells to any of the vital stains, and displays them suitably under the microscope,[3] it is obvious that many different procedures will be advocated. Having been interested in these cells for a number of years, and tried various methods, we have found the following to be the simplest and most satisfactory for clinical use. A stock 0.3 per cent solution of Brilliant Cresyl Blue (Grubler) in normal salt solution, is prepared in quantity sufficient to last several months, filtered and kept in the ice box to inhibit molds. (Saturated aqueous solutions have also been used). Before using, it is diluted with four (4) parts of a 2 per cent sodium oxalate solution in normal salt solution to one (1) of the stock stain and in this strength is useful for several days. (If Grubler's stain is not available, a 1:2000 aqueous solution of Janus Green is recommended as a stock solution, and similarly diluted before using. I have not yet found an American-made Brilliant Cresyl Blue that is satisfactory.) From a free flowing cut, a drop of blood is sucked into a pipette and quickly diluted about 1:10 with the stain (for this purpose a leucocyte pipette is convenient, taking double the amount of blood customary for a leucocyte count). After standing 10 to 15 minutes, this is well shaken and a wet coverslip prepara-

tion made from the contents, ringed with vaseline (to prevent drying and troublesome convection currents), and examined with the oil immersion lens. (Though for the most part easily visible in lower magnifications, some of the scantier reticula would inevitably be overlooked, unless the oil immersion is used.) This gives about 150 to 200 cells to a field, which can be reduced if desired by inserting a paper with a rectangular slit in the ocular. If reticulocytes are found to be frequent in the preliminary survey, the percentage is obtained by counting the number found in 500—or better 1000—erythrocytes, viewed in at least four different sections of the preparation. If rare—less than 1 per cent—truer results will be obtained if many more cells are examined, 10,000 or more. With good preparations, this can be done with reasonable accuracy by estimating the average number of cells per field, and counting the number of reticulocytes per field, until the requisite number has been examined. Especial care should be taken to prevent crenation, which makes the recognition of the reticulum more difficult and perhaps inhibits the entrance of the dye into the cell. If a permanent preparation is desired, more of the mixture can be spread on a slide, counterstained with a Romanowski stain, and mounted in the usual manner.

The above method seems to have some advantages over that of Vogel,[4] which has been advocated in some text books. It is not only simpler, but ensures a more representative sample. As the reticulocytes are said to have a different specific gravity from the normocytes, any method involving sedimentation would tend to introduce an error on this account.

A still simpler technic for clinical use employs a dry method with coverslips previously prepared with a dried film of the vital stain.[5] I have not found, however, that as high counts are obtained by this as by the wet method. In the haste necessary for making an even spread, it is possible that all the reticulocytes, and especially those with a very slight reticulum, are not properly stained.

The appearance of reticulocytes with Brilliant Cresyl Blue has already been so well described that it can be dismissed here with a reference to the accompanying sketch assembled from a slide prepared in the manner above described (Fig. 1). Attention should be drawn, however, to Key's observation that the form of the reticulum may vary greatly with the stain and method used, so that the familiar forms probably do not picture the structure as it occurs in the unaltered cell, where it probably exists as a diffuse substance. We have also noted that the reticulocytes tend to be larger than the normocytes, and if the slide is allowed to stand, more of them become shadow cells than do the normocytes. This would appear to be contrary to the usual opinion that the younger reticulocytes are more resistant than the adult cells to changes in tonicity of the containing fluid. We have also observed that even in preparations that are not overstained, some of the normalcytes assume a greyish green appearance, much darker than the rest. Whether this indicates a significant tinctorial difference in these cells, or whether it is an artefact due to some other factor, such as difference in osmotic pressure, we

are not in a position to decide. Metachromatic granules (staining red by this method) can occasionally be found.

### EXPERIMENTAL RETICULOSIS IN ARTIFICIAL PLETHORA

An opportunity was offered to study the percentage of reticulocytes in dog's blood during the production of experimental plethora by means of daily transfusions from dog donors over periods of several months.[6] This is similar to but more prolonged than Robertson's[7] experiments with rabbits and has afforded somewhat similar results.

In four dogs studied in this manner, the reticulocytes either vanished entirely or diminished to such a point (considerably less than 0.1 per cent) that the possibility must be considered that those found had been introduced with the slightly anemic donor's blood. The curve of one of these dogs is shown in Table I. The temporary rise in the reticulocyte count immediately

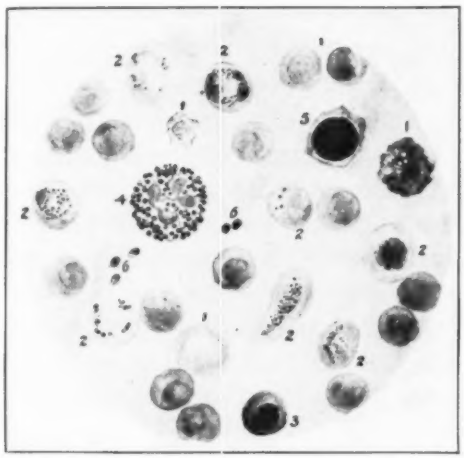

Fig. 1.—Sketch of selected blood cells, vitally stained with Brilliant Cresyl Blue; *1*, normocytes one pale, two others crenated; *2*, reticulocytes, showing granulofilamentous substance, appearing as a small or heavy network, or wreath; *3*, normoblast containing a reticulum; *4*, polymorphonuclear leucocyte, with highly refractile granules. *5*, lymphocyte with homogeneous nucleus and "glassy" protoplasm; *6*, blood platelets.

after transfusions were begun was found in another dog, and considered by us as probably due to bone marrow irritation.

A further record of the value of reticulocytes as an index of blood regeneration is shown in Table II, where dog 20-6, for reasons that we were unable to ascertain, developed a rapid and extreme hemolytic anemia six weeks after daily transfusions of 100 c.c. of whole blood had begun. Though the transfusions were continued without interruption, the hemoglobin count fell from 133 per cent (Newcomer) to 13 per cent in twenty-three (23) days, and the erythrocyte count from 10,000,000 to 650,000 per cu.mm. After ten (10) days, when the hemoglobin had reached 50 per cent, the reticulocytes began to increase and normoblasts and megaloblasts appeared in the peripheral circulation. The number of these fluctuated inversely with the hemoglobin count—the reticulocyte count being the more delicate and the more consistent—until after transfusions had been stopped, the reticulocyte percentage

arose to the extremely high level of 81 per cent. With the gradual recovery from anemia, the reticulocyte count fell again until after four (4) months both curves had reached almost normal levels.

The only instance that I am aware of in which such levels have been surpassed, is in Sappington's[8] rabbits made anemic with phenylhydrazine, where a reticulosis of more than 80 per cent was found in several instances and of 91 per cent in one instance, disappearing in ten days with return of the hemoglobin count to normal.

In dog 20-9 (Table I) a less severe anemia was present from the eighth to eleventh week, and during this period a mild reticulosis developed. It is to be expected that the degree of anemia at which the bone marrow will be sufficiently pushed to put forth an increased number of the relatively immature reticuloeytes will vary considerably in different individuals and condi-

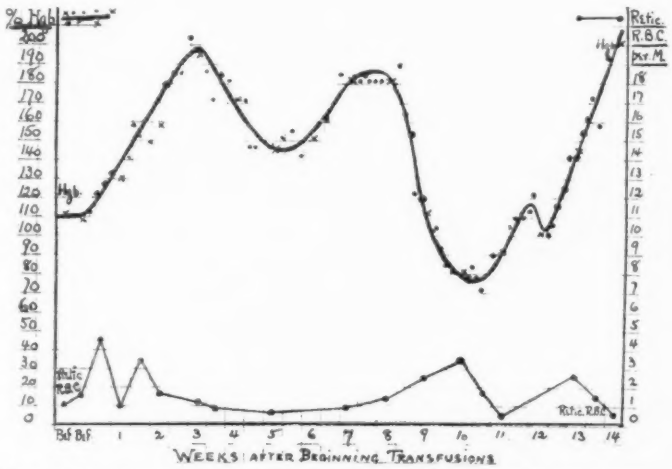


Table I.—Effect of daily repeated transfusions on hemoglobin and reticulocyte count. Dog. 20-9.

tions. With the continuance of transfusions the anemia in this dog was replaced by plethora and again the reticulocytes disappeared from the circulation.

## NORMAL STANDARDS IN MAN AND LABORATORY ANIMALS

As I have not been able to find any statement of the normal reticulocyte picture in the common laboratory animals, Table III has been prepared to show the averages and normal ranges in normal animals living under customary laboratory conditions. It will be noted that, especially in rabbits, guinea pigs and mice, the individual variation is considerable, and it is quite possible that other individuals of a different strain and living under different conditions, would show still further variations. Lee and Minot[9] have placed the average for man at 0.8 per cent, and in Wood's *Chemical and Microscopic Diagnosis* and Emerson's *Clinical Diagnosis* (5th edition) even higher figures are given. This is distinctly higher than I have found in normal adults, (0.3 per cent) but in view of the individual variations, the matter is sufficiently covered for practical purposes, if initial counts are considered abnormal only

if over 1 per cent or less than 0.1 per cent. Qualitatively it may be said that on the whole the reticulum tends to be large and heavy in the species that show the higher percentages. In the monkey it is especially delicate and feathery, while the human reticulocyte stands midway in these particulars, between those of the dog and cat, and those of the smaller animals.

### RETICULOSIS IN INFANCY AND DISEASE

The average reticulocyte count for the normal human adult has been discussed in the previous paragraph. Statements as to the normal averages for

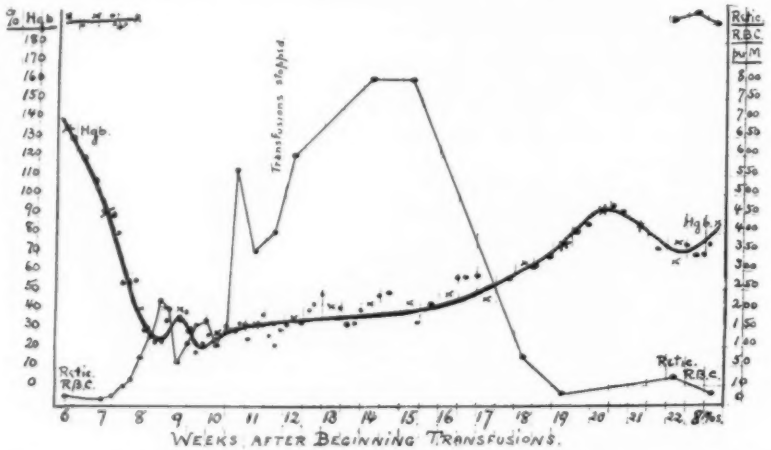


Table II.—Effect of daily repeated transfusions on hemoglobin and reticulocyte count, with supervention of anemia. Dog 20-6.

TABLE III

PERCENTAGE OF RETICULATED ERYTHROCYTES IN VARIOUS ANIMALS

| SPECIES | AVERAGE | NORMAL RANGE |
|---|---|---|
| Man | 0.3% | 0.1—0.8% |
| Monkey | 0.3% | 0 —0.8% |
| Dog | 0.6% | 0.1—1.4% |
| Cat | 0.2% | 0 —0.4% |
| Guinea pig | 3.0% | 1.0—4.0% |
| Rabbit | 2.0% | 0.6—2.8% |
| Mouse | 4.0% | 1 —6.0% |

infants, if given at all in text books of clinical pathology, are sufficiently at variance with the facts as I have found them easily to give rise to errors in diagnosis or prognosis. Thus it is usually stated that the average for normal infants is from 5 to 10 per cent, and even 20 per cent has not been considered abnormal. As will be seen in Table IV, I have not found any to exceed 5 per cent, or after the first twenty-four (24) hours to exceed 3 per cent. By the end of the first week, practically normal levels are reached, so that with these exceptions, the normal infant's reticulocyte picture is the same as that of adults. This roughly parallels the hemoglobin and erythrocyte curve for the same period. Cathala and Daunay,[10] the only others who appear to have studied the reticulocytes in early infancy, also found an increased num-

ber at birth, which reached normal levels before the end of the first week. In eight infants they found from 11,000 to 360,000 reticulocytes per cu.mm. at birth; 25,000 to 155,000 the first day; 5,000 to 60,000 the second day; "very rare" by the seventh day; and (with the exception of one icteric) "very rare" by the seventh day. Allowing for the transient erythrocytosis that occurs during the first week of life, this would give a ratio of from 2 to 60 reticulocytes per thousand at birth, 3 to 20 per thousand the first day, 1 to 9 per thousand the second day; "rare" to 3 per thousand the fourth day; and less than one per thousand thereafter; i.e., distinctly lower figures than in our series. Reticulosis, on the other hand, in common with other hematological changes, occurs more readily and to a greater degree in infancy than during adult life.

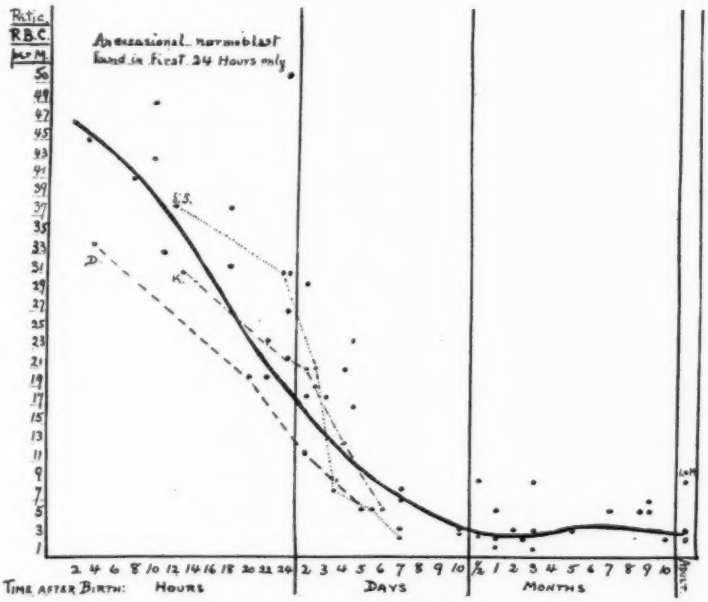


Table IV.—Curve showing number of reticulocytes per thousand during infancy. The solid line indicates the probable average curve; the broken lines denote a few characteristic individual curves.

Variations in the reticulocyte percentage in disease naturally depend on the intensity of the demand and the capacity of the bone marrow to respond. Thus is pernicious anemia, while there is usually a reticulosis of from 2 to 5 per cent during "blood crises" or periods of remission, this may rise to 10 or 15 per cent; while in periods of regression these cells may be completely absent. In the two forms of hemolytic jaundice, the reticulosis is out of all proportion to the severity of the anemia, being customarily as high as 10 and even 20 per cent. The reason for this increase, which is of diagnostic import, has not been determined. In secondary anemias, the reticulosis may be said to be roughly proportioned to the severity of the anemia, and according to Christian,[11] it is also present in the purpuras. In true polycythemia they are also said to be increased, which would support the view that there is an increased demand for erythrocytes in this condition. In aplastic anemia and

other forms of anemia due to decreased blood formation, these cells are diminished (reticulopenia) or absent.

## SUMMARY

1. Erythrocytes revealing a more or less extensive reticulum (granulofilamentous substance) by the methods of vital staining, may be conveniently designated "reticulocytes."

2. The reticulum is probably of protoplasmic origin, and indicates an intermediate stage between the erythroblast and the adult erythrocyte. A simple method for their recognition and estimation is described.

3. The greater delicacy of the tests for these cells and the greater constancy and delicacy of their variations in the peripheral blood, makes them more valuable criteria of the functional activity of the bone marrow than the study of polychromatophilia or nucleated forms.

4. In the blood of dogs made plethoric by repeated transfusions of blood, the reticulocytes diminished or disappeared entirely during the plethoric stage. With the occurrence of a hemolytic anemia, a reticulosis occurred, which in one instance reached 81 per cent.

5. The average percentage of reticulocytes and their normal range in man and the common laboratory animals is given.

6. The average curve of reticulosis during infancy is given, and the variations occurring in disease discussed.

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# SOME OBSERVATIONS ON BLOOD SUGAR AND THE ALLEGED GLYCOSURIA FOLLOWING OPERATIVE PROCEDURES ON THE THORACIC DUCT*

By Carl S. Williamson, M.D., Rochester, Minn.

RECENTLY in connection with certain studies on the thoracic duct observations were made on the blood sugar values and the alleged appearance of glycosuria following a fistula of the thoracic duct or ligation of the duct in the right pleural cavity.

Lepine and later Biedl maintained that glycosuria develops in a high percentage of instances following fistula of the thoracic duct or ligation of the duct in the pleural cavity. Biedl performed a large number of experiments on dogs. In some he ligated the thoracic duct and in others he made a fistula of the duct. A high percentage of all the animals developed glycosuria within a few hours after operation. The glycosuria continued in some instances until the death of the animal; in others it persisted for a few days and gradually disappeared. Biedl believed that the gradual disappearance of the glycosuria was due to the slow establishment of a collateral circulation of lymph. He was also able to lessen the severity of glycosuria by injecting lymph from a normal dog into the blood stream of a diabetic animal. Tuckett confirmed the work of Lepine and Biedl. Prior to the work of Lepine and Biedl, Gaglio found that if the thoracic duct was ligated or a duct fistula made and the pancreas was removed and glycosuria established, the severity of the symptoms diminished greatly; in fact the urine might even become sugar free. Lymph from a normal dog injected into a pancreatectomized dog also reduces the severity of the glycosuria. Tuckett, in a second publication, retracts his statement that glycosuria results from operative procedures on the thoracic duct and believes that the glycosuria produced by a fistula or ligation of the thoracic duct is such that it could be explained by either the anesthesia or trauma to the cervical nerves during operation.

If the foregoing statements are trustworthy, the importance of the lymph stream as a potential carrier of the internal secretion of the pancreas becomes self-evident. And in view of the fact that the data brought out heretofore are contradictory, it seems advisable to repeat the work.

The data in the present study have been obtained from observations on the dog. All operative procedures were conducted under ether anesthesia, and with sterile technic. The operative technic for fistula of the thoracic duct was that of Biedl with Mann's modifications. Following operation the

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ducts were massaged to keep them open; otherwise they often closed within a week. By careful treatment it was possible to maintain a good flow of lymph from two to three weeks after operation. For ligation of the thoracic duct portions of the sixth and seventh ribs were resected and an opening into the right chest made. The lung was walled back with moist sponges

TABLE I

BLOOD SUGAR VALUES BEFORE AND AFTER OPERATION

| DOG | DATE | TIME | BLOOD SUGAR PER CENT | REMARKS |
|---|---|---|---|---|
| E 443 | 3-29-22 | 8:30 a.m. | 0.107 | Before operation |
| | | | | Thoracic duct ligated |
| | | 9:30 a.m. | 0.205 | After operation; ether hyperglycemia |
| | | 4:15 p.m. | 0.10 | |
| | 3-30-22 | 8:15 a.m. | 0.10 | |
| | | 4:30 p.m. | 0.073 | |
| | 3-31-22 | 8:30 a.m. | 0.077 | After death |
| | | | | Thoracic duct fistula |
| E 788 | 11-1-21 | 4:25 p.m. | 0.167 | After operation; ether hyperglycemia |
| | 11-2-21 | 1:30 p.m. | 0.096 | |
| | 11-5-21 | 8:15 a.m. | 0.10 | |
| | 11-7-21 | 3:00 p.m. | 0.103 | |
| | | | | Thoracic duct fistula |
| E 787 | 11-1-21 | 3:15 p.m. | 0.117 | After operation; ether hyperglycemia |
| | 11-2-21 | 1:30 p.m. | 0.10 | |
| | 11-3-21 | 10:20 a.m. | 0.150 | |
| | 11-5-21 | 8:15 a.m. | 0.15 | |
| | 11-7-21 | 3:00 p.m. | 0.073 | |
| E 770 | 10-26-21 | 2:00 p.m. | 0.87 | Before operation |
| | | | | Thoracic duct fistula |
| | | 2:50 p.m. | 0.155 | After operation; ether hyperglycemia |
| | 10-27-21 | 8:45 a.m. | 0.107 | |
| | | 5:00 p.m. | 0.105 | |
| | 10-28-21 | 8:30 a.m. | 0.10 | |
| | 10-29-21 | 8:30 a.m. | 0.079 | |
| | 10-31-21 | 8:15 a.m. | 0.12 | |
| | | 4:05 p.m. | 0.111 | |
| | | 4:30 p.m. | 0.107 | |
| | 11- 1-21 | 10:45 a.m. | 0.144 | |
| | 11- 5-21 | 8:30 a.m. | 0.10 | |
| | 11- 7-21 | 3:00 p.m. | 0.079 | |
| | 11-10-21 | 9:00 a.m. | 0.09 | |
| | 11-11-21 | | 0.083 | |
| | 11-12-21 | | 0.092 | |
| E 568 | 4- 5-22 | 3:25 p.m. | 0.113 | Before operation |
| | | | | Thoracic duct ligated |
| | | 4:35 p.m. | 0.197 | After operation; ether hyperglycemia |
| | 4- 6-22 | 8:30 a.m. | 0.137 | |
| | | 4:30 p.m. | 0.122 | |
| | 4- 7-22 | 4:30 p.m. | 0.109 | |
| | 4- 8-22 | 9:00 a.m. | 0.153 | |
| | 4-10-22 | 10:00 a.m. | 0.125 | |
| E 661 | 4- 5-22 | 2:45 p.m. | 0.092 | Before operation |
| | | | | Thoracic duct ligated |
| | | 3:10 p.m. | 0.082 | After operation |
| | 4- 6-22 | 8:30 a.m. | 0.119 | |
| | 4- 7-22 | 4:30 p.m. | 0.113 | |
| | | 8:30 a.m. | 0.109 | |
| | | 4:15 p.m. | 0.103 | |
| | 4- 8-22 | 8:45 a.m. | 0.10 | |
| | 4-10-22 | 1:00 p.m. | 0.085 | |
| | 4-11-22 | 8:30 a.m. | 0.092 | |

TABLE I—CONT'D.

BLOOD SUGAR VALUES BEFORE AND AFTER OPERATION

| DOG | DATE | TIME | BLOOD SUGAR PER CENT | REMARKS |
|---|---|---|---|---|
| E 569 | 4- 5-22 | 2:00 p.m. | 0.15 | Before operation<br>Thoracic duct ligated |
| | | 2:55 p.m. | 0.254 | After operation; ether hyperglycemia |
| | 4- 6-22 | 8:30 a.m. | 0.187 | |
| | | 4:30 p.m. | 0.187 | |
| | 4- 7-22 | 8:30 a.m. | 0.103 | |
| | | 4:15 p.m. | 0.136 | |
| | 4- 8-22 | 8:15 a.m. | 0.153 | |
| | 4-10-22 | 9:30 a.m. | 0.129 | |
| | 4-11-22 | 8:30 a.m. | 0.109 | |
| E 142 | 2-29-22 | 10:00 a.m. | 0.121 | Thoracic duct fistula |
| | | 4:15 p.m. | 0.121 | |
| | 3-30-22 | 8:15 a.m. | 0.136 | |
| | | 4:15 p.m. | 0.136 | |
| | 3-31-22 | 9:00 a.m. | 0.09 | |
| | | 4:15 p.m. | 0.102 | |
| | 4- 1-22 | 8:30 a.m. | 0.11 | |
| | 4- 3-22 | 8:30 a.m. | 0.11 | |
| | | 4:00 p.m. | 0.094 | |
| | 4- 4-22 | 8:45 a.m. | 0.12 | |
| | | 4:00 p.m. | 0.121 | |
| | 4- 5-22 | 8:30 a.m. | 0.121 | |
| | 4- 6-22 | 8:15 a.m. | 0.118 | |
| | | 4:30 p.m. | 0.112 | |

and the duct ligated in two or more places in its course upward from the diaphragm. In making a fistula of the thoracic duct or in ligating it, an anesthenometer was used to prevent asphyxia should the pleura be opened accidentally in making the duct fistula or intentionally in ligating the duct.

Before operation urine was collected and blood was taken for sugar estimations. A second specimen of blood was taken at the end of the operation, and afterwards specimens of urine were taken once or twice daily. Subsequent specimens of blood for sugar estimation were taken twice daily. Benedict's modification of the Lewis-Benedict method was used for estimating sugar in the blood, and Fehling's method for qualitatively testing for sugar in the urine was employed. Care was taken to collect all data during the time when the ducts were discharging freely. If a duct began to show signs of closing, blood and urine examinations were abandoned.

A fairly wide variation in blood sugar values is shown in the accompanying tabulation. The occasional postoperative increases are not sufficient to be significant. The average postoperative blood sugar value, disregarding the reading obtained immediately after operation is practically the same as the value immediately prior to operation. In some instances it is even a little lower than the preoperative value.

At the time these data were obtained, interesting facts were noted on the relation of the sugar of blood and lymph; these observations will appear later.

Findings with regard to sugar in the urine were uniformly negative before and after operation.

## CONCLUSIONS

1. Practically no changes in the blood sugar level were observed following operative procedures on the thoracic duct, (thoracic duct ligation or thoracic duct fistula).

2. Glycosuria did not develop in any of the animals in our experiments.

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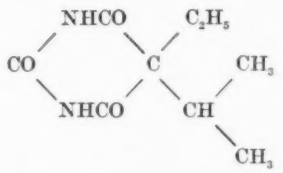
# THE PHARMACOLOGICAL ACTION OF ISOPROPYLETHYL-BARBITURIC ACID*

By D. E. Jackson, Ph.D., M.D., Cincinnati, Ohio

THE drug used in the experiments performed in the investigation herein described was very kindly given to me by Dr. Lambert Thorp who has made the compound synthetically. Chemically the substance is isopropyl-ethylbarbituric acid, having the following formula:




It is a white, flocculent powder having a melting point of 197° C. It is made from ethylisopropyl-malonic-diethyl ester and urea by condensation in the presence of sodium ethylate at 105° C. The substance is slightly soluble in cold water, more soluble in hot water and readily soluble in ammonia water, alkaline carbonates and caustic alkalies, forming with the latter the corresponding salts which are readily soluble in alcohol and water, but insoluble in ether. From the aqueous salt solutions, the free acid, melting at 197° C. may be reprecipitated by addition of dilute mineral acids. The compound is readily soluble in ether and in alcohol, but insoluble in aqueous solutions of sodium bicarbonate. Microscopically the substance consists of fine white needles which are very stable in the presence of acids. On boiling with sodium hydrate it decomposes into ammonia, sodium carbonate and the sodium salt of ethylisopropyl malonic acid.

Within the limits of medicinal dosage the action of this compound is confined to the central nervous system, and especially to the cerebrum. This action can perhaps best be demonstrated by the administration to dogs by stomach of increasing doses of the acid which has been dissolved in water by the addition of a sufficient quantity of sodium carbonate. Under these circumstances it is found that the drug acts as an efficient and reliable somnifacient. The action of the drug comes on in from twenty to forty minutes, and the symptoms appear to be produced by an overpowering desire on the part of the animal to lie down quietly and go to sleep. At first the animal shows some slight ataxy and unsteadiness in its actions, but when kept well aroused, it appears to readily regain to a considerable extent control over its movements. If let alone, however, it soon lapses into a somnolent state again and falls fast asleep. So far as it is possible to judge from

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the appearance and reactions of the animal this condition bears a very striking resemblance to natural sleep. With moderate dosage the respiration and circulation appear to be practically uninfluenced by the drug. The breathing is regular and of normal depth, and the heart beat is full, regular and apparently of normal force. When let alone, the animal lies quietly and apparently sleeps soundly. But if it be slightly aroused by loud noises or shaking, it slowly opens its eyes, raises its head, gazes blankly about, and then very frequently yawns exactly as a very tired, sleepy child would do, and then, if not disturbed further, it soon lays its head down again and goes

Fig. 1.

back to sleep, as if nothing concerned it except a strong natural desire to sleep and rest. If, however, the animal be more vigorously aroused, even though the sleep had been very profound, it will be found that the animal may appear to wake up and try to get on its feet and move about. The movements are weak, unsteady and incoördinated at first, but with persistent efforts the animal may gain a very considerable degree of control over its movements in a short time and may, in fact, finally be able to get up and move about the room, apparently of its own accord. If not further disturbed, however, it soon lies down quietly again and goes to sleep.

As the dose is increased, the action of the drug tends to appear earlier, and the sleep deepens into a coma from which the animal can be aroused only with great difficulty, or in the deeper stages, not at all. With very large doses, this coma may last for twelve or eighteen hours and the animal may then recover. In some cases animals lived more than forty-eight hours after being given enormous doses, but finally died, perhaps partly from exhaustion and failure to properly take nourishment, as well as from the action of the drug. In the milder cases of prolonged sleep the animals sometimes appear spontaneously to wake up partially, and then they may stretch their limbs, sigh, or very often yawn, and possibly try to move about. Slight muscular tremors frequently are produced by very large doses. These tremors generally first appear in the shoulders or fore limbs, but later may develop in the hind limbs. The tremors appear to be due to a mild stimulation

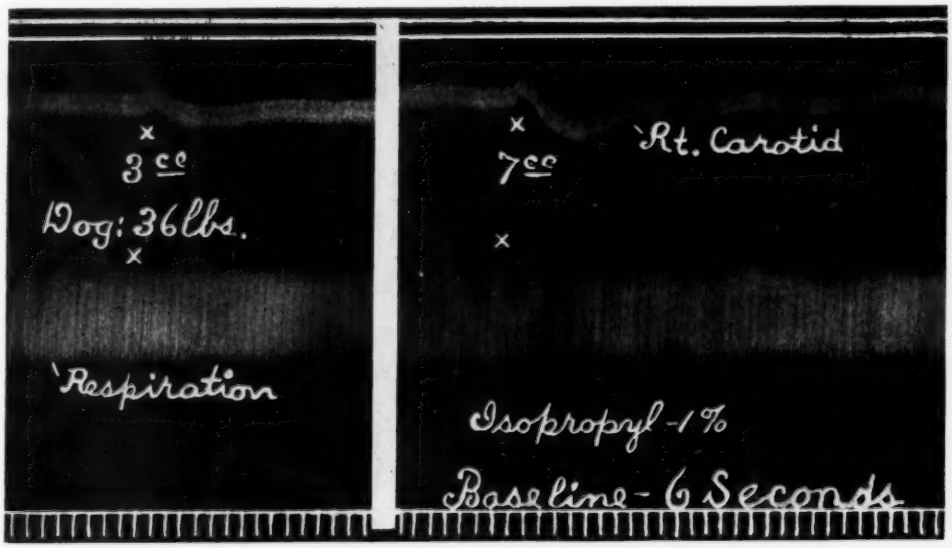


Fig. 2.

of some portions of the spinal cord. They are irregular and intermittent in character, and are usually mild in type. Sometimes twitching may be noticed in the neck or jaw muscles. The tremors may appear at a fairly early stage in the action of the drug in some cases, but they are apparently of but little importance so far as the general action of the drug is concerned. They are of interest, however, because other drugs of this general type have been reported as occasionally causing generalized convulsions. I have watched for this action closely with this drug, but in no case has the slightest tendency toward the development of generalized convulsions been observed. Occasionally in very deep and prolonged sleep from the action of the drug the animal may at times temporarily breathe very rapidly, and occasionally what appeared to be some slight expiratory difficulty has been noted. These actions probably are the result of some slight indefinite stimulation or depression of the medullary, or upper cord, regions by the drug.

There is no bronchoconstriction produced by the drug so far as its direct action on the bronchioles is concerned as is shown in Fig. 1 which represents the blood pressure and lung action in a pithed dog. The excursions in the upper tracing show no change in amplitude when 25 c.c. of one per cent solution of the drug were injected into the femoral vein. (For method of making these lung tracings see Jour. of Pharmacol. and Exper. Therap., 1914, v, 479; also (in detail), Jackson: Experimental Pharmacology, 1917, St. Louis, C. V. Mosby Co., p. 287). Fig. 2 also shows very obviously that ordinary doses have but little or no effect on the respiratory apparatus including the respiratory center in the medulla. In Fig. 2, two separate records are

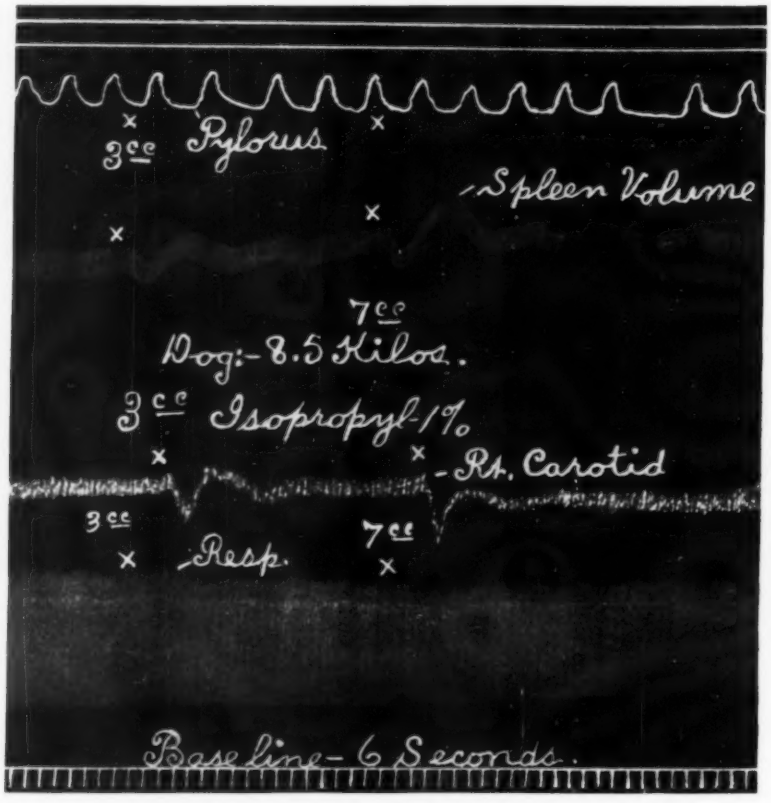


Fig. 3.

joined together to show the effects on the blood pressure and respiration of increasing doses of the drug when injected intravenously. In the first record 3 c.c. of one per cent solution (30 mg. or ½ grain) of the drug were suddenly injected into the femoral vein. This represents approximately a small therapeutic dose for man. It is seen that the respiration is entirely unaffected as indicated by the respiratory tracing. The blood pressure shows first a very slight rise (from the mechanical increase of the volume of fluid as the solution is suddenly injected into the vein) and then a slight fall. This fall, however, is very insignificant and in two or three minutes the pressure is again back to normal as seen in the second record in which an injection of

7 c.c. is given. This second injection represents 70 milligrams, or proportionately almost twice the therapeutic dose for man. The slightness of the effect on the respiration and the early recovery of the blood pressure indicate that the toxicity of the drug is low. Fig. 3 illustrates the action of the drug on the pyloric sphincter, the spleen volume, the blood pressure and the respiration of a small dog (8.5 kilos). The first injection of 3 c.c. exercises extremely little influence on the regular normal peristaltic contractions of the pylorus. This is significant as throwing some light on the action of the drug on the gastrointestinal system. It would appear that the stomach and intestines are almost entirely uninfluenced by ordinary doses of the compound. The spleen volume is slightly increased by this injection while the respiration is depressed to a very slight degree. The second injection of 7 c.c. (70 milligrams) represents proportionately from two to three times

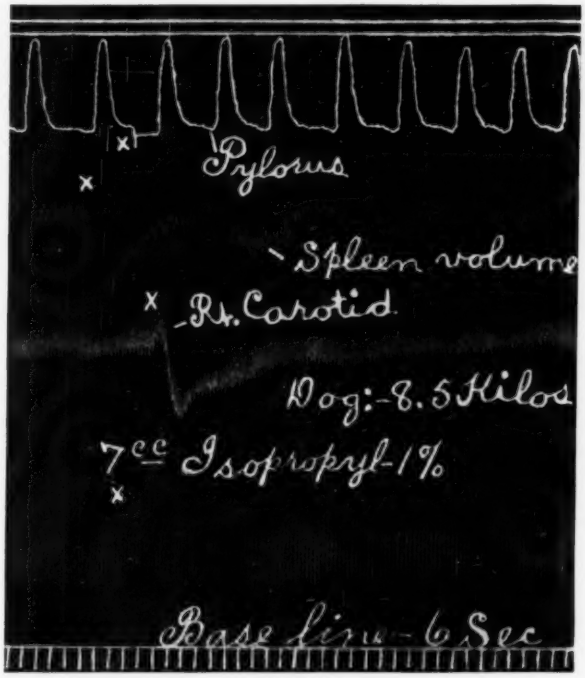


Fig. 4.

the ordinary therapeutic dose by stomach for man. Both of these injections cause slight temporary falls in blood pressure, but this soon again regains almost its normal level. The spleen shows further enlargement by the second injection while the amplitude of the respiratory tracing is somewhat further decreased. There is also detectable a very slight slowing of the respiratory movements near the end of the tracing.

Fig. 4 also shows that the pyloric contractions are not affected by the drug, while the spleen volume tracing takes on an undulating appearance indicating a series of very slight contractions and dilatations. These are perhaps entirely without importance. The respiration shows no immediate

change, but near the end of the tracing some increase in the amplitude is seen. This is of interest as being a very early indication of a phenomenon which becomes much more marked after very large doses; viz., a considerable increase in depth of the respiratory movements as the respiration becomes progressively slower. In this tracing the blood pressure falls sharply following the rapid injection of the drug into the femoral vein, but the pressure soon regains its normal level again, thus indicating that no permanent injury has been done to the circulatory organs.

Fig. 5.

Fig. 5 shows the action of a dose of 15 c.c. (150 milligrams, 2½ grains) on the spleen volume, blood pressure and respiration. A sharp and rather extensive, but very transient shrinkage in spleen volume occurs. This is perhaps mainly secondary to the sudden, brief drop in blood pressure. But very soon, however, the pressure rises almost to normal again. The depressing effects of the drug on the respiration in this case are more marked and more lasting than those shown in the previous records. This depressing effect on respiration becomes more and more evident with increasing doses and is the final cause of death in fatal cases.

Figs. 6 and 7 should be considered together. They both show the direct action on the heart and on the blood pressure. The animal was etherized, the chest opened and under positive artificial respiration the pericardium was opened and a myocardiograph was attached directly to the heart. The tambour bowl of the myocardiograph was connected by rubber tubing to a recording tambour which wrote on the smoked surface of the kymograph paper. A record of the normal heart tracing and blood pressure was taken and then (Fig. 6) an injection of 3 c.c. of one per cent solution of the drug was injected into the femoral vein. This dosage was approximately proportional to the therapeutic dose in man. It is seen that the heart beat is

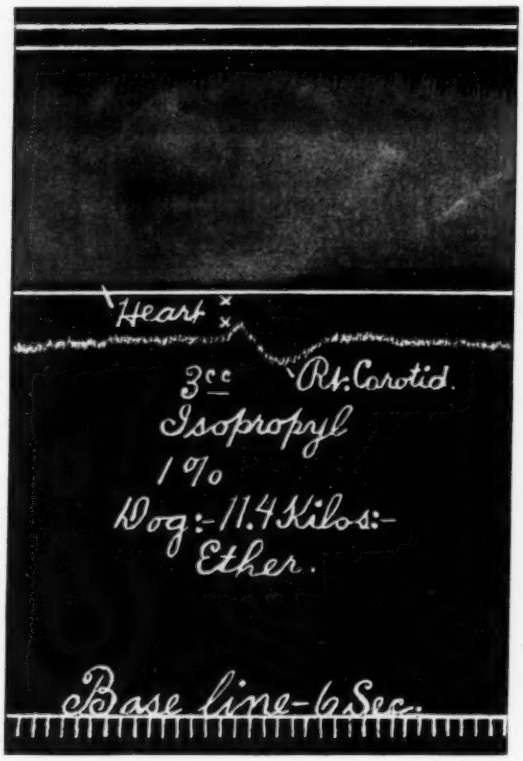


Fig. 6.

but little affected by the drug when the entire dose is injected suddenly directly into the circulation, in which case the compound reaches the heart in its greatest concentration almost at once. In addition the heart in this case was already under the influence of sufficient ether to maintain the animal quietly in a state of anesthesia. This slight preliminary depression from the ether should render the heart more susceptible to the isopropyl compound if it possessed any very marked depressant action on the cardiac muscle. Since the drug under these conditions exercises almost no action in therapeutic quantities on the heart, we may safely conclude that this organ lies almost entirely outside the sphere of action of the drug when given in any

such quantities as would be used medicinally. It may be added in passing that the vagus inhibitory mechanism is entirely unaffected by the drug.

Fig. 7 shows three further injections of enormous quantities into the same animal. Here it is seen that larger doses tend to weaken and decrease the systolic action (down stroke) of the heart while the diastole (up stroke) is slightly increased. But the tendency of the blood pressure to rise again after the fall produced by each injection is very obvious. No such proportionate quantities as these could, of course, be used therapeutically.

I have regularly found experimentally that an animal etherized in the beginning will soon pass under the influence of this compound when a few large injections are given, and then the ether can be entirely removed from

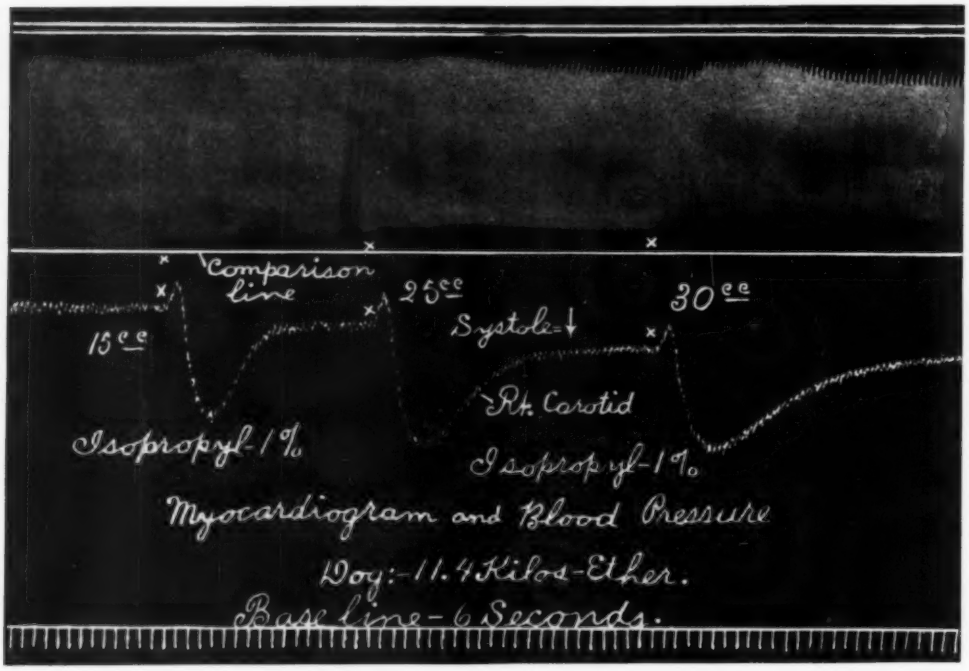


Fig. 7.

the animal. Under these circumstances, the blood pressure and respiration remain in excellent condition. It is often especially advantageous to give one or two fairly large injections of the drug to quiet down an animal which does not take ether well, but continues to breathe rapidly and irregularly and to have slight convulsive seizures, but in which a slight excess of ether tends to immediately stop the respiration or greatly lower the blood pressure. In these cases the reflexes can be quieted down and the animal can be reduced to a calm and satisfactory condition by two or three fair sized injections of the drug. The ether can then be continued without further difficulty.

The final cause of death under this drug is central respiratory paralysis. This is well shown in Fig. 8 in which the respiration stopped spontaneously

after repeated, large injections of the drug. Just before the final respiratory failure the blood pressure had risen to a considerable height as a result of the asphyxial stimulation of the vasomotor centers in the medulla. The kidney volume had also constricted markedly. These two actions show that the circulatory apparatus, both centrally and peripherally, was in very good condition at the time of paralysis of the respiratory center. This is still further shown by the prompt recovery of the heart beat and the blood pressure soon after artificial respiration was started (at the crosses marked "On"). The kidney volume also soon showed evidences of a tendency of the vasculature of this organ to return toward the normal state. The blood pressure does not rise so high after the artificial respiration is started as it had been before the normal respiration ceased, probably because of the asphyxia

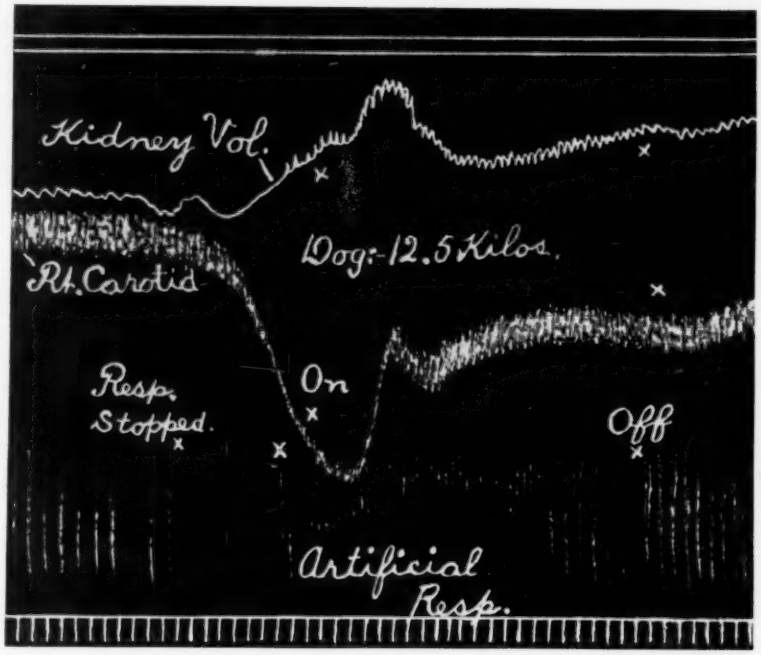


Fig. 8.

which preceded the stoppage of the respiration. After a time it was found that the animal could again breathe spontaneously and then the artificial respiration was stopped. The animal does not recover, however, under these conditions, but after a few minutes again ceases to breathe and dies of central respiratory paralysis. But with care the animal may be repeatedly revived after the respiration ceases, thus showing that artificial respiration is the chief remedy needed in cases of severe poisoning with the drug, but in such cases it is to be noted that the drug is apparently excreted rather slowly.

I have made no special observation regarding the method or form of elimination of the drug from the body, but by the analogy of related compounds, it is very probable that the substance is partly oxidized and partly excreted by the kidneys. I have not noted any special diuresis in dogs fol-

lowing administration of the drug, but it is possible that such action may occur in other animals and perhaps to a slight extent in man. Diuretics, such as caffein, should probably be given in cases of poisoning in man. And respiratory stimulants would also be indicated. From experiments on intact animals it seems obvious that the circulation in general remains in good condition until the respiration has become exceedingly feeble or failed entirely.

I have made no clinical observations under the drug myself, but a considerable number of observations by others (Drs. Lurie, L. A., Cook, R.H., et. al.)* have shown that the dose (of the acid) which is required to produce a mild sedative effect in the average adult is 2 grains. If deep sleep is desired and the patient is in a highly excited or delirious state, 3½ grains may be given. These doses may be increased or repeated in case the symptoms call for a more marked depression. Or a large dose (4 or 5 grains) may be given initially (e.g., to maniacal patients) and then small doses (2 grains each) may be given once or twice daily thereafter. These small doses keep the patient calm and enable him to secure quiet, refreshing sleep (Cook, R. H.). The drug (in the form of the sodium salt) may also be administered hypodermically in 2 grain doses two or three times per day. Of the sodium salt (which is very soluble in water) the dose by mouth is two to four grains.

From the therapeutic action of the drug it seems obvious that man is more susceptible to the hypnotic effects of the compound than are dogs. In these animals I have found that the dose beyond which recovery does not occur is in the neighborhood of one grain to the pound of weight of the animal. This would indicate that an average patient of one hundred and fifty pounds weight might survive a dose of nearly ten grams or one hundred and fifty grains. I am inclined to suspect, however, that the fatal dose in man would be smaller than this amount, unless the stomach should be emptied very soon after the drug had been swallowed. The lower degree of susceptibility possessed by dogs, as compared with man, probably holds only in the case of the higher, psychical areas of the cerebrum. And in this case man's higher psychical development probably mainly accounts for his increased susceptibility over that of the dog. So far as the heart and the other circulatory organs and the respiratory apparatus are concerned, it is probable that no great difference exists between man and the dog.

*Personal Communications.

# THE EFFECT OF WARM AND COLD WEATHER ON THE BLOOD CATALASE*

By W. E. Burge, and J. M. Leichsenring, Chicago, Ill.

IT IS known that oxidation in warm-blooded animals is increased by a fall in the external temperature and decreased by a rise until ordinary room temperature is reached when the stimulating effect of cold on metabolism is reduced approximately to its minimum.[1] This investigation is an attempt to determine the mode of action of cold in increasing oxidation in the body. During the past several years we have carried out experiments in which oxidation in animals was increased as well as decreased in practically every known way. Invariably it was found that whenever oxidation was increased, catalase, an enzyme possessing the property of liberating oxygen from hydrogen peroxide, was also increased and whenever oxidation was decreased, catalase was decreased. Hence we naturally turned to this enzyme in our attempt to find an explanation for the increase in oxidation brought about by cold weather.

The animals used were rabbits. Catalase determinations were made by adding 1 c.c. of the blood diluted 1 to 3 with 0.9 per cent sodium chloride to 200 c.c. of neutral hydrogen peroxide and the amount of oxygen liberated in 10 minutes was taken as a measure of the catalase content of the blood.

In Table I is shown a comparison of the catalase content of the blood of rabbits in the summer and winter. It may be seen that the blood catalase

TABLE I

The figures after rabbit 1, 2, 3, 4, etc., indicate cubic centimeters of oxygen liberated from neutral hydrogen peroxide in ten minutes by one c.c. of the rabbit's blood diluted 1 to 3 with 0.9 per cent sodium chloride.

| RABBIT | BLOOD CATALASE OF ILLINOIS RABBITS | |
|---|---|---|
| | SUMMER (74° F.) | WINTER (30° F.) |
| 1 | 850 | 1290 |
| 2 | 750 | 1560 |
| 3 | 700 | 1080 |
| 4 | 520 | 940 |
| 5 | 975 | 1050 |
| 6 | 800 | 970 |
| 7 | 920 | 1445 |
| 8 | 940 | 1220 |
| 9 | 1040 | 1300 |
| 10 | 830 | 1175 |
| 11 | 760 | 1330 |
| 12 | 815 | 1045 |
| Average | 825 | 1200 |

*From the Physiological Laboratory, University of Illinois.
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in the summer when the average temperature was 74° F. was much lower than that of rabbits in the winter when the average temperature was 30° F. The average amount of oxygen liberated by 1 c.c. of the diluted blood of the rabbits in the summer was 825 c.c. while it was 1200 c.c. in the winter. These rabbits were kept in the open, winter and summer, and fed on a uniform diet of hay, oats and bread.

Catalase determinations were made of the blood of rabbits for each month from August to April inclusive. These rabbits were also kept in the open, during winter and summer, and fed on a uniform diet. The results obtained

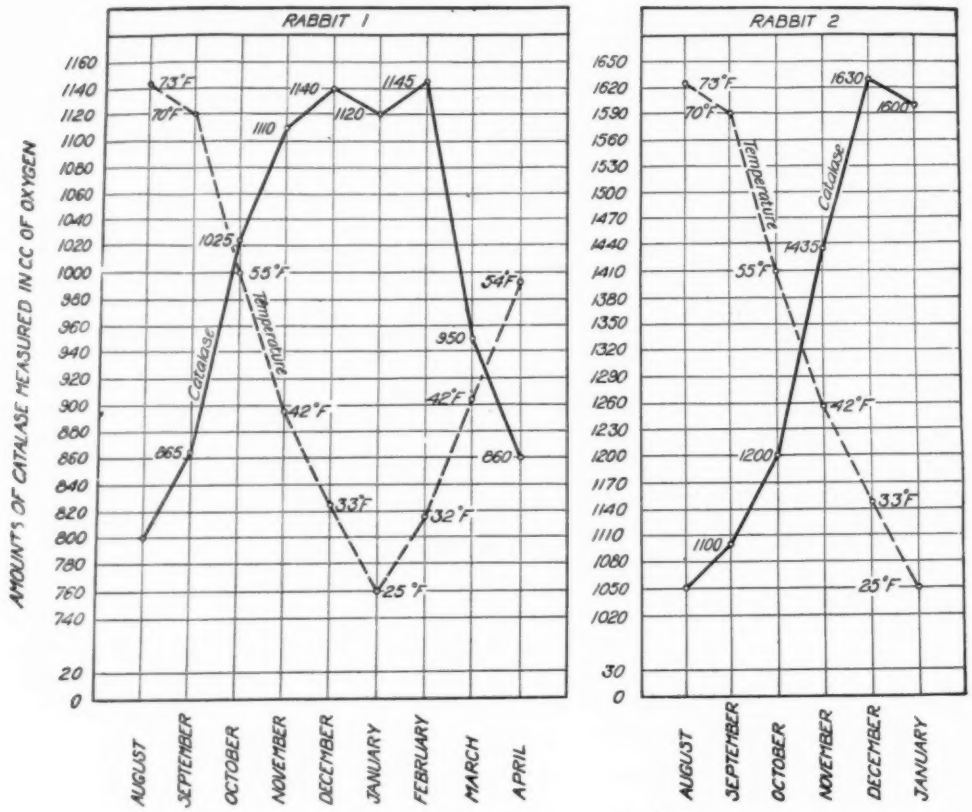


Fig. 1.—Curves showing the effect of a fall in temperature from August to January on the blood catalase of rabbits and of a rise in temperature from January to April.

from two of these rabbits, which are typical, are shown in Fig. 1. It may be seen that the temperature fell from 73° F. in August to 25° F. in the following January, and that there was an increase in catalase during this period as is indicated by the fact that in August, 1 c.c. of the diluted blood liberated 800 c.c. of oxygen from hydrogen peroxide in 10 minutes, whereas in January, 1 c.c. of the diluted blood liberated 1120 c.c. It may be seen further that when the weather began to grow warmer during the months of February, March and April, there was a corresponding decrease in catalase.

In Table II is shown a comparison of the blood catalase of Illinois and

Louisiana rabbits in December. It may be seen that the temperature in Illinois was 33° F. while it was 70° F. in Louisiana. It may also be seen that the blood catalase of the rabbits of Illinois, where it was cold was considerably higher than those of Louisiana where it was warm. The average amount of oxygen liberated by the blood of the five Illinois rabbits was 1014 c.c. while the blood of the Louisiana rabbits liberated only 759 c.c.

In Fig. 2 is shown the effect on the blood catalase of bringing rabbits in the month of December from Louisiana, where it was warm, to Illinois,

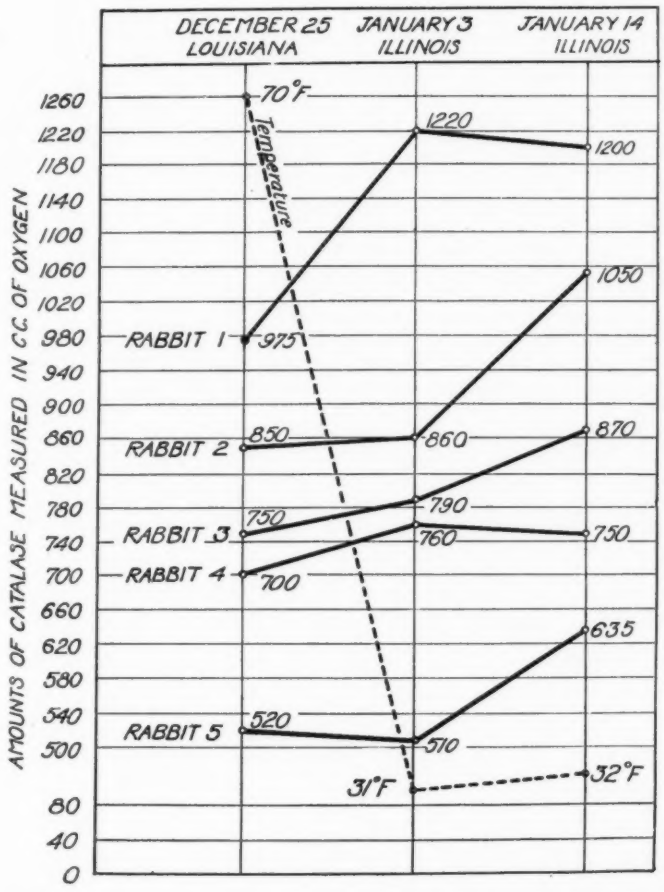


Fig. 2.—Curves showing the effect on the blood catalase of bringing rabbits from Louisiana to Illinois in December.

where it was cold. The average temperature for the month up to December 23 was 70° F. in Louisiana. One c.c. of the diluted blood of No. 1 Louisiana rabbit liberated 975 c.c. of oxygen from hydrogen peroxide in 10 minutes, Rabbit 2, 850 c.c., Rabbit 3, 750 c.c., Rabbit 4, 700 c.c., Rabbit 5, 520 c.c. The rabbits were brought to Illinois and kept there from December 25 to January 3, when catalase determinations were again made. It may be seen that the catalase of the blood of Rabbit 1 had increased a great deal, while Rabbits 2, 3, 4, and 5 had increased very little. From January 3 to January 14, how-

TABLE II

The figures after rabbit 1, 2, 3, 4, 5, indicate cubic centimeters of oxygen liberated from neutral hydrogen peroxide in ten minutes by one c.c. of the rabbit's blood diluted 1 to 3 with 0.9 per cent sodium chloride.

| RABBIT | BLOOD CATALASE OF ILLINOIS AND OF LOUISIANA RABBITS | |
|---|---|---|
| | ILLINOIS (33° F.) DECEMBER | LOUISIANA (70° F.) DECEMBER |
| 1 | 1080 | 750 |
| 2 | 940 | 700 |
| 3 | 1050 | 975 |
| 4 | 970 | 850 |
| 5 | 1030 | 520 |
| Average | 1014 | 759 |

ever, the blood of all the rabbits showed a considerable increase in catalase with the exception of Rabbit 4, which showed only a small increase.

## SUMMARY

The blood catalase of Illinois rabbits is lowest in the summer when the weather is hottest and highest in the winter when the weather is coldest.

As the weather grows colder, passing from summer to fall and winter, the blood catalase gradually increases, and passing from winter to spring and summer when the weather is growing warmer, the blood catalase gradually decreases.

The blood catalase of Louisiana rabbits in December is much lower than that of rabbits at the same time in the colder climate of Illinois. However, if the Louisiana rabbits are brought north to Illinois in the winter, the blood catalase rapidly increases.

The increase in oxidation in warm blooded animals brought about by cold weather is attributed to an increase in catalase and the decrease in oxidation brought about by warm weather is attributed to a decrease in catalase.

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# STUDIES ON GASTRIC AND DUODENAL ULCER: THE RELATION OF EPIGASTRIC HERNIA TO GASTRIC ULCER—A CLINICAL AND EXPERIMENTAL STUDY*

By Jacob Meyer, M.S., M.D., and A. C. Ivy, M.S., Ph.D., Chicago, Ill.

EPIGASTRIC hernia is frequently encountered by the surgeon and internist. It is present in about 1 per cent[1, 2] of all patients examined. From Hall's experience with soldiers only about 20 per cent of the cases of epigastric hernia have symptoms and they are those in which omentocele is present, bowel being present in the hernia only in a very small percentage of the cases.

The symptoms complained of are either obscure abdominal pain or discomfort, a feeling of "dragging" in the epigastrium, or the gastric ulcer syndrome.

The literature on this subject records fifty-four cases of epigastric hernia with gastric symptoms. Of these fifty-four cases, 30 are reported as having had symptoms comparable to those of the gactric ulcer syndrome, but ulcer is proved (blood in feces and vomitus, revealed by operation) only in seven of these cases. (We are liberal when we state that ulcer was proved in seven cases.) In other words, the literature reports that 13 per cent of cases of epigastric hernia with gastric symptoms had a concomitant gastric ulcer.

The early observers Ury,[3] Strauss,[4] and Cohnheim,[5] and more recently Hall,[2] call attention to the possibility of epigastric hernia symptomatology being mistaken for gastric ulcer. But we are of the opinion that with the present methods of history taking and gastroenterological diagnosis a differential diagnosis should not be difficult. On the other hand, we do believe that the marked similarity and relationship between epigastric hernia symptomatology and the gastric ulcer syndrome should be emphasized and kept in mind by the surgeon and internist in the differential diagnosis of gastric ulcer.

Our interest in this condition was aroused by the following case: S. T., age twenty-two, clerk, male, complained of pain in the epigastrium, belching and constipation. Pain was severe, "hunger-like" and gnawing in character, and occurred regularly about two hours after meals, was located in the epigastrium and relieved by alkalies and posture. Physical examination was negative, except for the presence of a small, soft, globular mass about two inches above the umbilicus in the median line. This mass was not reducible and no impulse was obtained on coughing. Gastric analyses showed a free acidity of forty units and a total acidity of one hundred units. A diagnosis of epigastric hernia possibly associated with gastric ulcer was

*From the Department of Physiology, Loyola University School of Medicine.
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made and the patient was advised to enter the hospital for further observation. As this was refused, the patient was placed under ulcer treatment as recommended by Sippy. Six weeks later, with no apparent improvement, the patient returned and entered the hospital for examination. At this examination the gastric analyses showed a free acidity of twenty-seven and a total acidity of eighty-one units. Fluoroscopic studies and serial plates of the stomach showed no evidence of any lesion in the stomach.

In view of these observations, an operation was advised to correct the hernia. The operation by Dr. B. F. Lounsburg revealed a small globular mass (one and a half centimeters in diameter) of properitoneal fat that was continuous with the omentum (omentocele) which protruded through a small defect in the linea alba and was adherent to the parietal peritoneum. Gross inspection of the stomach showed no evidence of ulcer of the stomach. The appendix was removed because of the presence of fecal concretions, but histological examination showed it to be normal. The patient was placed on a full diet for a period of a week after the operation. Two years have elapsed since the operation and the patient is still free from all symptoms.

Because of the apparent comcomitance of gastric ulcer and epigastric hernia with omentocele, the latter is alleged to be the cause of the gastric ulcer. Kelling[6] offers the hypothesis that the tension exerted by the omentocele "irritates the sensory nerves of the stomach wall, a secondary stimulus of the sympathetic ganglion results, impulses are originated which produce a narrowing of the arteries; the circulation in the submucosa accounts for the development of the ulcer, or prevents the healing of one when originated by other causes," Strauss[4] reports the opinion that the continual pull exerted by the hernia on the stomach wall causes the development of the ulcer. Ury[3] states that the hernia appeared to be a factor in the development and course of the ulcer in his cases. Soper[7] expresses the opinion that the hernia does not cause the ulcer but prevents the healing of an ulcer produced by some other factor. Hall[2] believes that "long continued reflex irritation of the stomach by the omental 'drag,' so that hyperacidity and ulcer follow, as in chronic appendicitis, produces the ulcer."

Since it is alleged that epigastric hernia with omentocele is an etiological factor of gastric ulcer and since we have in this condition one that can be easily and accurately duplicated and put to experimental test, we thought it worth while to make an experimental study of epigastric hernia in the dog as it is related to gastric ulcer.

## EXPERIMENTAL PROCEDURE

An epigastric hernia was produced by the following method in a series of dogs: The abdomen was opened in the mid-line by a two inch incision downward from the tip of the xiphoid process. Gastric omentum about one-half of an inch from the pyloric sphincter and one inch from its attachment to the greater curvature of the stomach was picked up and sutured to the external sheath of the rectus outside the linea alba in such a manner that a "tug" or pull would be exerted on the stomach by every contraction

of the stomach and that free herniation of the adjoining omentum would occur. One inch of the incision was closed by interrupted sutures leaving a defect of about one inch in the abdominal wall. The subcutaneous fat and fascia was then drawn together by interrupted sutures and the edges of the skin approximated by a subcuticular suture. Fourteen dogs were so operated. After operation the animals were examined, x-rayed and autopsied.

## SUMMARY OF RESULTS

Of the fourteen dogs operated on, thirteen showed a definite and typical epigastric hernia of the gastric omentum (by examination and autopsy) so situated that there would be a "tugging" action exerted on the greater curvature of the pyloric portion of the stomach about one-half to one inch from the pyloric sphincter. In four dogs this condition was present for six months and in others for one to two months. We x-rayed the four dogs which were kept six months to ascertain whether there was a deformity of the stomach caused by the "tugging" action of the herniated omentum and to prove definitely whether or not this tugging actually existed in our experimental condition. Slight deformity of the greater curvature was observed in two of the animals and in all of them a "tugging" action could be demonstrated by pulling on the herniated omentum, thereby causing marked deformity of the greater curvature at the site of attachment. In six animals a definite pocket or pouch formation associated with dense fibrous bands was found at autopsy on the greater curvature of the stomach one inch from the pyloric sphincter at the site of attachment of the herniated portion of gastric omentum. Bone fragments were found in the "gastric pocket" of one of these animals, the stomach being otherwise empty. From these results and observations we are convinced that a definite "tugging" action was exerted on the stomach and some deformity of the stomach was produced by the experimental epigastric hernia.

## DISCUSSION

Since we have noticed in our experimental animals with epigastric hernia the occurrence of a pocket formation in the wall of the stomach, we might suggest that such a pocket may be considered as a locus minoris resistentiae and a potential site for an ulcer. This would be of greater significance in man than in the dog because of the upright position that man assumes. Also such a factor might lead to the production of an ulcer in man and not in the dog, because gastric ulcer occurs very infrequently in dogs[8] and it is difficult to experimentally produce a chronic ulcer in a healthy dog. On the contrary, such a pocket formation has never been reported to occur in man. Only two cases have been examined fluoroscopically, however, our case and one of Hall's cases.

Since the above suggestion is only speculation, we interpret our negative results as demonstrating that epigastric hernia is not a direct etiologic factor in gastric ulcer. If it were a causative factor, we should at least have got an acute ulcer. If Kelling's or Strauss' hypotheses were true, an

ulcer should have occurred because the mechanisms referred to by them existed in our dogs. Whether such a reflex irritation or vasoconstriction as the various hypotheses infer actually exists is physiologically very questionable.

We prefer to believe that the concomitance of epigastric hernia and gastric ulcer is a coincidence. We explain the percentage (13 per cent) of concomitant occurrence of epigastric hernia and ulcer by the supposition that cases of concomitance of the two conditions are reported because of their interest, whereas cases with epigastric hernia without proven ulcer are not reported because such cases are relatively common.

Our experiments have no bearing on Soper's opinion that the hernia causes a delay in or prevents the healing of an ulcer from some other cause. In our opinion this hypothesis has much in its favor and we expect to put it to an experimental test. Soper's case which failed to respond to treatment until the hernia was operated and repaired, strongly supports his contention.

Our case showed what may be considered by some clinicians as hyperacidity, in the view of the observations of Carlson,[9] Ivy[10] and the contributions of Refuss and Hawk, such findings may be within the physiological variation of normal persons. It is conceivable that an epigastric hernia may cause reflex disturbances which in turn might alter gastric secretion. We are convinced, however, that this does not occur because in our experience with Pavlov pouch dogs in which several epigastric hernias involving the omentum have accidentally occurred, no abnormal acidity or secretion has been observed. It is also to be kept in mind that it has not been demonstrated that hyperacidity is an etiologic factor of gastric ulcer; in fact Dragstedt[11] has shown that hyperacidity is not a causative factor of gastric ulcer.

The character of the pain or distress in our case interested us very much because a gastric ulcer was not present. The pain was almost identical with the pain of gastric ulcer, occurring one to two hours after meals, dull, gnawing and intermittent in character, located in the epigastrium and relieved by soda and food. Cohnheim ascribed the genesis of these pains to the tension exerted on the herniated omentum brought about by the vigorous contractions of the stomach which occur intermittently. We concur in this opinion and believe that it is supported by the fact that alkalies will relieve both types of pain, the pain of gastric ulcer and of epigastric hernia with omentocele, the alkalies relieving the pain according to Carlson[12] by causing an inhibition of the vigorous tonic contractions, which begin one to two hours after meals and continue until the stomach is empty then to disappear until a hunger period ensues. This opinion is further supported by the fact that coarse food excites and enhances the pain in cases of epigastric hernia (Soper[7]).

## SUMMARY

We are unable to demonstrate experimentally in dogs that epigastric hernia with omentocele is a causative factor of gastric ulcer. We tacitly suggest that the tendency to a local pocket formation in the wall of the

stomach brought about by the "tugging" of the omentocele may be an etiologic factor of gastric ulcer in man. We prefer to believe that concomitant gastric ulcer and epigastric hernia in man is coincident and not that the hernia has a direct etiologic relationship to the ulcer. Epigastric hernia does not cause hyperacidity, as the gastric findings in such cases are within the normal variation. We are convinced, as are all others who report cases of epigastric hernia with gastric symptoms, that operation is definitely indicated.

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# THE RELATION OF FIBROSIS AND HYALINIZATION TO LONGEVITY IN CANCER*

## (A Study of 194 Cases)

William C. MacCarty, M.D.,† Rochester, Minnesota

IN June, 1921, I published a series of observations on the relation of local lymphocytic infiltration and cellular differentiation to the length of postoperative life in gastric carcinoma.[1] While making those observations no special attention was paid to the relation of fibrosis and hyalinization to postoperative life. It was not done because both fibrosis and hyalinization

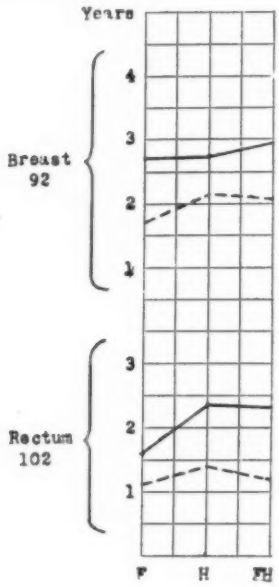


Fig. 1.

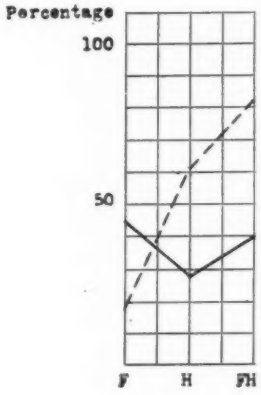


Fig. 2.

Fig. 1.—Average length of postoperative life with and without factors.
Solid line, duration of life of cases with the factor. Dotted line, duration of life of cases without the factor.

Fig. 2. Percentage increase of postoperative life with factors checked against postoperative life without factors.
Dotted line, rectum. Solid line, breast.

are rather infrequent in the series of gastric carcinomas which has come under my observation. There are, however, two organs in which these factors (Figs. 1 and 2) are more frequent and it was thought that a study of these might throw some light on the problem of the defensive mechanism in cancer. Two series of patients, all of whom had died of recurrence or metastasis following radical resection, were studied:

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The following generalizations may be made:

1. The frequency of fibrosis in association with cancer of the breast and rectum is practically the same in both organs.

2. The frequency of hyalinization in association with cancer of the breast is greater than in cancer of the rectum.

TABLE I

OBSERVATIONS IN 194 CASES

| | BREAST | RECTUM |
|---|---|---|
| Cases | 92 | 102 |
| Average length of postoperative life | 2.5 years | 1.47 years |
| Frequency of associated fibrosis | 68 per cent | 75 per cent |
| Frequency of associated hyalinization | 52 per cent | 2 per cent |
| Frequency of associated fibrosis and hyalinization | 52 per cent | 2 per cent |
| Average length of postoperative life with associated fibrosis | 2.72 years | 1.53 years |
| Average length of postoperative life without associated fibrosis | 1.87 years | 1.29 years |
| Average length of postoperative life with associated hyalinization | 2.81 years | 2.33 years |
| Average length of postoperative life without associated hyalinization | 2.21 years | 1.44 years |
| Average length of postoperative life with associated fibrosis and hyalinization | 2.89 years | 2.33 years |
| Average length of postoperative life without associated fibrosis and hyalinization | 2.05 years | 1.28 years |

3. When fibrosis is present in association with cancer of the breast and rectum the postoperative length of life is increased 34 per cent.

4. When hyalinization is present in association with cancer of the breast and rectum the postoperative length of life is increased 40 per cent.

5. When the two factors (fibrosis and hyalinization) are present in combination in association with cancer of the breast and rectum the postoperative length of life is increased over 56 per cent.

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# *LABORATORY METHODS*

## A SIMPLE TECHNIC FOR THE PREPARATION OF A SPINAL (HEADLESS) ANIMAL*

By Reginald Alex Cutting, M.A., Ph.D., Chicago, Ill.

VARIOUS technics have been devised for the preparation of spinal animals for purposes of research in physiology and pharmacology, as well as demonstration before classes in these subjects; they fall into two general classes, (1) those which depend upon the use of surgical procedures, such as pithing and gross decapitation, and (2) those which depend upon the use of drugs, as, for instance, the injection of chloroform into the vertebral artery (Jackson) or carotid artery (Luckhardt) or directly into the fourth ventricle (McGuigan and Heinekamp). The latter methods make a special appeal to pharmacologists not only because the procedures involved are relatively simple and the results generally satisfactory, but more important still, the attainment of the end by pharmacological means is intrinsically a mental satisfaction. The physiologist, on the other hand, and especially the physiological pedagogue, hesitates to use drugs in procedures in which the same end can be attained without them since their use unnecessarily obscures an already complicated picture. The use of chloroform, or any other drug, as an agent for the destruction of the higher centers of the central nervous system is, accordingly, from the physiologist's point of view open to this general criticism, which in case of experiments where beginners in physiology are concerned, may amount to the difference between pedagogical success and failure, and, furthermore, to the specific criticism that the action of the drug introduced in any of the ways already described cannot be properly controlled; even with careful technic the injection of chloroform into the cervical arteries may not be without cardiac effects, and if the drug be injected directly into the fourth ventricle it becomes impossible to say with certainty the exact level to which the effect may be extending, indeed, in the latter case, it becomes somewhat a matter of chance whether so important a center as that governing respiratory movements is or is not destroyed by the mere mechanical passage of the needle. Not only so, but the matter of technical simplicity which the pharmacological methods favor is apt to be more apparent than real since one must take into consideration the fact that the operation of tracheotomy, as a minimum, must be performed for purposes of artificial respiration whatever the method used.

As between pithing and gross decapitation the former method has the dis-

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advantage that, while almost as radical as the latter surgically, the site of the operation is, in this case, more or less obscure both during and after the operation and, further, the procedure may be inhumane since it leaves the part of the animal central to the point of section essentially uninjured unless the blood supply to the head be cut off or the brain itself destroyed, procedures in the first case amounting technically to decapitation and in the second case involving uncontrollable hemorrhage if thoroughly performed.

The process of injecting starch granules into the cephalic circulation as a mechanical means of destroying the brain substance is ingenious, but has little besides its ingenuity to recommend it.

Taking into consideration these factors, and many others which might be mentioned if space permitted, the original operation of decapitation (Sherrington) seems to be by far the best technic for routine work on the spinal animal and should be the method of choice for most special purposes as well. The decapitate preparation is not difficult to make; the following method has been used by the author for three or four years and has proved simple and serviceable; it entails so little manipulation that, in the case of the common laboratory animals, cats, dogs, rabbits, etc., the entire operation including the maintenance of anesthesia can be performed by a single individual in less than half an hour.

The animal should be preferably a male and in good physical condition. Ether anesthesia is induced in the usual manner and is maintained with the ether bottle after tracheotomy; the incision for the latter should extend from the lower margin of the cricoid cartilage nearly to the suprasternal notch. Starting from the laryngeal end of this incision two secondary skin incisions are carried upward and backward to encircle the neck and meet dorsally in the median line at the level of the atlanto-occipital articulation; in making these incisions one should avoid cutting so deeply as to injure the superficially placed external jugular veins.

The cervical margin of the skin, including the deep fascia, is now dissected up and turned back for several centimeters, thus exposing the musculature of the neck. The closed blunt points of a pair of long curved hemostatic forceps are next plunged under the anterior musculature at the level of the third cervical vertebra, entering them just behind the external jugular vein on the left, working them through just anterior to the vertebral column, and bringing them out on the right side also just posterior to the external jugular vein; the end of a strong waxed cord is now caught between the jaws of the forceps, and the latter is withdrawn, thus pulling the cord through under the musculature. The cord is tied tightly about the muscle mass without including the trachea; this manoeuvre shuts off the blood supply through the two carotid arteries and the external and internal jugular veins.

The hemostats are inserted at the same point as before, but this time they are worked through the musculature posterior to the vertebral column and are brought out in the median line at the level of the third cervical vertebra; a ligature is passed and tied in the manner previously described. The corresponding muscle group on the right side is also tied off. In tying these ligatures care should be taken to draw the cord very tight, for their function is to

cut off blood supply; a convenient form of knot is the common "slip-knot" secured with a "half-hitch."

At this point 5 c.c. of a 1-10,000 solution of adrenalin in normal salt solution is injected subcutaneously in the flank region, the ether supply is cut off, and artificial respiration introduced, and at the same time heat is supplied to the body by means of an electric heating pad, a bank of incandescent electric lights, hot water bottles, or the like.

The vertebrals are now to be occluded; to do this the hemostats are inserted successively beneath each of the muscle groups already tied, drawing the cord through in such a way as to encircle the spinal column; the ligature is tied tightly, working it down firmly between the second and third cervical vertebrae.

The apex beat will be feeble after this procedure, but should be readily palpable; in case it is not the last ligature is to be loosened and 3 c.c. more of the adrenalin solution injected; in this case the ligature is again tightened at the end of five minutes.

At the end of five minutes, if the heart still continues to beat properly, the head is severed from the body above the ligatures, the incision being carried through the vertebral column between the atlas and occiput; this can be done quickly with the aid of an amputating knife.

After wiping away the blood which drains back from the head and stopping any small hemorrhages which may occur from the cervical stump, the latter is covered with gauze or cotton and the skin flaps are brought together and secured with hemostats.

The blood-pressure rises slowly; reflexes may be expected to return gradually at the end of twenty minutes.

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## ARTIFACTS IN BLOOD CULTURE PLATES SIMULATING COLONIES*

By William Thalhimer, M.D., Milwaukee, Wis.

In a blood culture taken recently with a new Roux syringe, colony-like structures developed which simulated exactly colonies of Streptococcus viridans. It required a great deal of labor to determine that the structures which appeared to be colonies were artifacts. This is the second time that I have had this experience and I have seen this same phenomenon twice in blood cultures taken at Mt. Sinai Pathological Laboratory, New York City, which Dr. E. P. Bernstein kindly showed me. The cases from which my own blood cultures were taken were febrile cases where there was a possibility of positive blood cultures being present. These apparently positive blood cultures consequently caused considerable anxiety until they were proved to be negative. I, therefore, considered it sufficiently important to determine the nature of these struc-

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tures which simulated bacterial colonies, and, perhaps, others who have been similarly misled may profit by the results of my experience.

My first experience was with a blood culture taken at the University of Virginia Laboratory of Pathology in 1910. It was only after my second experience that I recalled that the previous apparently positive blood culture had also been taken with a Roux syringe. In both instances, the syringe was new and it occurred to me that perhaps some minute particles in the new syringe became mixed with the blood and gave rise to the development around them of colony-like structures. The Roux syringe has a rubber plunger and a glass barrel encased in metal. Upon the new rubber there is a thin layer of yellowish or cream colored powder, the exact constitution of which I do not know, but which, I believe, contains sulphur. It occurred to me that the fine particles of this powder might cause the phenomenon referred to. The pseudocolonies, in each instance, developed slowly. A few appeared in twenty-four hours, a moderate number in forty-eight hours and all were present by seventy-two hours. By transmitted light on the blood agar plates, the colonies appeared as minute, slightly greenish specks. Immediately around these specks there was an area of clearing from one-half to one millimeter in width and immediately around this a second area twice as wide as the first one which was definitely greenish in tint and which shaded gradually into the surrounding medium. The pseudocolonies were target-like in appearance. By reflected light, these structures were dull gray in color. Under the low-power of the microscope, the speck at the center of these pseudocolonies was translucent in appearance and had a bluish color. The speck was irregular in outline and did not have the structure of a bacterial colony. Many colonies were fished from the plates in each blood culture and numerous smears from these were examined and many subcultures were made on various types of media (bouillon, agar, glucose serum agar, blood agar, etc.). In no instance was growth obtained in the subcultures nor were bacteria demonstrated in the smears. In both blood cultures the bouillon flasks remained sterile.

The deductions made above as to the cause of the colony-like artifacts were proved to be true by the following simple experiment:

A new rubber plunger from a Roux syringe was boiled in normal saline solution and some of the powder of the surface was scraped off with a sterile platinum loop and mixed with a tube of human blood agar at 45°. This was poured into petri dishes and controls were also poured of the blood agar containing none of the scrapings. The plates were then compared and the control plate remained sterile and showed no structures at all resembling colonies. In the plate of blood agar mixed with scrapings from the rubber plunger, colony-like structures identical with those described above appeared in small numbers in twenty-four hours. In forty-eight hours, hundreds of these structures appeared. Many of these structures were fished, subcultures made and smears were also examined. No bacteria could be demonstrated by either of these methods.

Many blood cultures have since been made with the original syringe after

the powder of the surface had been thoroughly cleaned off and no colony-like artifacts have appeared in any of the plates.

It is well recognized that minute specks may gain entrance into a blood culture, and into the blood plates which are poured, and that sometimes these specks will cause structures very similar in appearance to colonies to develop around them. These specks undoubtedly vary in constitution, so that various types of specks may give rise to this phenomenon. In the two instances recorded here the powder from the surface of the rubber plunger of the syringe was undoubtedly responsible for the pseudocolonies. In view of this, it is hoped that the findings recorded above will not prove uninteresting.

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## PRESERVATION OF COMPLEMENT SERUM WITH 25 PER CENT SODIUM CHLORIDE*

By M. W. Lyon, Jr., M.D., and Vera B. Trager, South Bend, Indiana.

THE preservation of complement serum with sodium chloride has been advocated by many workers. Kolmer, Matsunami and Trist† in an elaborate piece of work have reviewed the subject of complement preservation and performed numerous experiments to determine suitable methods for its preservation. They showed that the most successful method of preserving complement serum is by the addition of 17 per cent sodium chloride and keeping the serum cold.

It occurred to us that the addition of more sodium chloride might be more effective and that the greater dilution required to make the solution of complement and salt isotonic would not require the addition of so much, if any, isotonic salt solution in complement-fixation tests to make a satisfactory bulk for the reagents used. Apparently the largest amount of sodium chloride that serum will hold in solution is 25 per cent. One cubic centimeter of complement serum containing this amount of salt diluted to 30 c.c. yields a 1:30 dilution of complement in an isotonic salt solution. The sodium chloride strength is 0.833 per cent instead of the more usual 0.850 per cent. If diluted 1:29.4 the resulting percentage of salt is 0.85 per cent.

Complement serum preserved in this manner proved so satisfactory that it has been used by us for over a year in complement-fixation work.

The bottle of salted serum, along with control serums, is wrapped in a towel and placed in contact with the ice of the refrigerator. A thermometer inserted in the folded towel registers between 4° and 5° C. By neglect of iceman on one occasion the folded towel containing a bottle of some fresh complement serum was left on the floor of the ice box closet and remained at room temperature for 24 hours. Its hemolytic activity was apparently unaffected as shown by subsequent titrations. Being curious to see how well salted comple-

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†Am. Jour. Syph., October, 1919, iii, 513-540.

ment serum would keep at room temperature, the next lot of complement serum, collected by heart puncture from eight guinea pigs and salted to 25 per cent, was divided into two parts. A small portion was kept at room temperature and titrated on the regular Wassermann test days along with the bulk of the serum kept on the ice. The table shows the hemolytic unit of the diluted complement serums on successive days, using 0.1 c.c. of a 10 per cent suspension of chicken cells. The chicken blood is obtained from a poultry butcher twice a week and obviously never comes from the same chicken. The cells of individual chickens apparently vary in their resistance to specific amboceptor and complement so that it not infrequently happens that a succeeding titration shows a smaller complement unit than a preceding one. Not every lot of complement serum keeps its activity as well or as long as the one shown in the table.

After the eighteenth day the complement kept at room temperature appeared so weak that no further titrations were made with it. On this day a two millimeter loop of the salted serum kept on ice and another of that kept at room temperature were planted on agar slants. In the case of the room temperature serum, about 100 colonies of a Gram-positive nonpigment forming staphylococcus developed in the incubator. In the case of the ice box preserved serum no colonies developed after 24 hours' incubation, but after several days at room temperature seven colonies of a Gram-negative deep yellow pigment producing bacillus developed. On the twenty-eighth day similar cultures were made. The room kept serum showed essentially the same result as on the eighteenth day. In the case of the ice box kept serum only two colonies developed after several days' maintenance at room temperature, one the same yellow producing bacillus and the other a large nonsporogenous Gram-positive bacillus producing no pigment. The presence of bacteria in preserved complement serum is probably a factor in its deterioration.

No special tests to determine the loss of specific fixation or the acquirement of nonspecific fixation properties were made by us, but an experience of a year's use of complement serum saturated with sodium chloride have yielded results in the Wassermann tests done with three antigens in agreement with clinical findings, with histories, and in a number of instances with the results of Wassermann tests made by others. In laboratories having comparatively few Wassermann tests to do it is probably better to use a preserved complement serum obtained from several animals rather than fresh complement obtained from a single animal.

## SUMMARY

Complement serum preserved with 25 per cent sodium chloride, that is to point of saturation, and kept on ice retains its activity for hemolysis with specific amboceptor with comparatively little change for from three to four weeks and is satisfactory in performing the Wassermann test. The same salted serum kept at room temperature retains such activity for about 10 days. Bacteria can exist in serum saturated with sodium chloride even though such serum is kept on ice. It is not improbable that the presence of bacteria in preserved complement serum is a factor in its deterioration.

Table showing hemolytic activity of complement serum preserved with 25 per cent sodium chloride, some kept on ice and some kept at room temperature, diluted 1:30.

Days since collecting and adding 25 per cent sodium chloride and smallest quantities of diluted serum needed to hemolyze 0.1 c.c. of 10 per cent sensitized chicken erythrocytes.

| | 4 | 7 | 11 | 14 | 18 | 21 | 24 | 28 days |
|---|---|---|---|---|---|---|---|---|
| Salted serum kept on ice | 0.2 | 0.15 | 0.15 | 0.2 | 0.15 | 0.2 | 0.2 | 0.3 c.c. |
| Salted serum kept at room temperature | 0.2 | 0.15 | 0.25 | 0.35 | 0.5 | | | |

## A GASOMETRIC METHOD FOR THE DETERMINATION OF UREA NITROGEN IN THE BLOOD*

(Preliminary Communication)

By A. Mirkin, Ph.D., New York City

UREA can give off under suitable conditions three different gases: ammonia, nitrogen and carbon dioxide. An accurate method based upon the determination of either one of them would be superior to any colorimetric or titrimetric method from the standpoint of the clinician as such a method would greatly simplify and shorten the procedure. This accounts for the popularity which the so-called hypobromite method is still enjoying, although the inaccuracy and unreliability of the latter have been sufficiently demonstrated. Even still less accurate gasometric methods, as the decomposition with Millon's reagent or with nitrous acid, were used to some extent for the "quantitative" determination of urea.

There is a tendency to attribute the persistency with which many clinicians refused, and some still refuse, to heed the chemical objections raised by chemists to such methods to a lack of chemical training among medical men. This is not quite true. In his work, the clinician must have at his disposal methods capable of yielding quick and reliable results and the procedure must be as simple as possible. Between two methods of which the one is cumbersome and slow, and the other simple and quick, the clinician is liable to choose the latter even if its accuracy is questioned from many sides.

In many cases of nephritis, the urea nitrogen is so far above the normal that the inaccuracy of the method could not disguise it. And for a long time, the determination of urea nitrogen was considered only in nephritic cases. But not all the nephritic cases have abnormally high urea nitrogen; and we know, at the present, that a moderately high urea nitrogen is met with in many other diseases which cannot be characterized as those of renal involvement.

The present technic of urea nitrogen determination based upon the decomposition of urea with the aid of urease and colorimetric or titrimetric

*From the Department of Laboratories, Beth Israel Hospital, New York City. Dr. Max Kahn, Director.

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determination of the ammonia formed leaves no excuse to the clinician for using the notoriously inaccurate hypobromite method, even if the latter is braced up, remodeled and introduced in its new shape as capable of yielding results within the limits of permissible error.[1] We do not wish to question the accuracy of Andresen's figures, but even with the most perfected apparatus for the determination of evolved nitrogen, the fact still remains that the reaction between urea and hypobromite is neither quantitative nor specific; i.e., other nitrogenous compounds present in the blood gave off nitrogen under the same conditions as urea.

While the urease-aeration-(or distillation) Nesslerisation technic deprives the hypobromite method of *raison d'être*, it suggests itself a gasometric method which has one of the advantages of the former—specificity of urea—and at the same time is simpler and quicker. Instead of determining the $NH_3$ of the $(NH_4)_2CO_3$ formed through the action of urease, the carbon dioxide can be determined as well by using the well-known Van Slyke's apparatus for the determination of the carbon dioxide combining power or a more suitable modification. As the blood contains a certain amount of carbon dioxide, this would have to be determined first and deducted from the amount found by decomposing the bicarbonate of the blood incubated with urease.

Partos[2] describes an apparatus for the determination of the carbon dioxide content of the $(NH_4)_2CO_3$ formed from urea through incubation with urease. With this apparatus he determined the urea in the urine and checked up the results with the titration method. The smallest amount of urea which he determined in the urine was 7.56 mg. He also determined the urine in a pure aqueous solution, the smallest amount used in the determination being 1.65 mg. The amount of urea nitrogen in 1 c.c. of normal blood is almost 0.1 of the last named figure, but we saw no reason why even such a small amount could not yield correct results and we proceeded to satisfy ourselves to that effect after a careful scrutiny of available literature failed to disclose that this had already been tried by somebody else.

The following contains a description of the technic we used and of the results obtained so far.

*Technic*

| A. Test for total $CO_2$ | B. Test for preformed $CO_2$ |
|---|---|
| 2 c.c. plasma | 2 c.c. plasma |
| 1 c.c. urease | 1 c.c. $H_2O$ (free from $CO_2$) |

Incubate A and B for about ten minutes at 45-50° C. Cool with running water to room temperature. Run exactly 1.5 c.c. of A into Van Slyke's apparatus, add 0.5 c.c. of distilled water free from $CO_2$ and determine the carbon dioxide in the usual way. Do the same with B. For calculation, use the following formula:

$$Ur_{100} = 63.6\ V_1 - V_2)\ Wpt.$$

$Ur_{100}$ = the amount of urea nitrogen per 100 c.c. of blood.
$V_1$ = Volume of $CO_2$ yielded by A.
$V_2$ = Volume of $CO_2$ yielded by B.
Wpt. = Weight of 1 c.c. of $CO_2$ at the temperature and pressure prevailing during the test.

$$63.6 = \frac{NH_2.CO.NH_2}{CO_2} \times \frac{N_2}{NH_2.CO.NH_2} \times 100 = \frac{2800}{44} = 63.6$$

[1]Andresen, Gad: Biochem. Ztschr., 1919, xcix, 1.
[2]Partos: Biochem. Ztschr., 1921, ciii, 292.

In Van Nostrand's Chemical Annual (1919, page 100) will be found a table giving the respective weights of $CO_2$ at different temperatures and pressures.

It is needless to say that care in carrying out the procedure is of the utmost importance. The apparatus must be absolutely airtight, the stopcocks well greased, and all the precautions recommended when carrying out gasometric determinations must be painstakingly observed. The reading must be taken as soon as the level of the mercury in the calibrated part of the apparatus becomes steady. One must raise the hand holding the large bulb with mercury so that it should, as nearly as possible, be on the same level with the mercury in the calibrated part of the apparatus. Not more than 0.01 c.c. of caprylic alcohol should be added to prevent foaming; an excess makes the exact reading more difficult.

COMPARISON OF RESULTS OBTAINED BY BOTH THE GASOMETRIC AND COLORIMETRIC METHODS. MGMS. OF UREA NITROGEN IN 100 C.C. OF BLOOD.

| Colorimetric Method | Gasometric Method |
|---|---|
| 19.1 | 19.5 |
| 18.4 | 18.9 |
| 25.7 | 26.3 |
| 30.1 | 29.6 |
| 49.0 | 49.6 |
| 55.0 | 56.0 |

I wish to thank Dr. M. Kahn, director of the chemical laboratory, Beth Israel Hospital, for his courteous and ever-ready help extended to me during the work carried out.

## A SUBSTITUTE FOR THE LONG ROLL KYMOGRAPH*

BY FELIX P. CHILLINGWORTH, M.D., BOSTON, MASS.

THE necessity of producing several long roll kymographs for student use and the high cost of the same resulted in the writer's evading the issue by having the following type of large drum made.

This drum fits accurately the Harvard kymograph motor, and having a circumference of 90 cm. and a height of 30 cm. results in a greatly increased area of smoked writing surface. The ends of the drum are made of light wood, through which the sleeve passes. The height of the drum permits the use of a wide kymograph paper which has distinct advantages, namely—by an adjustment upward or downward of the drum, the writing point level can be readily changed, which does away with the prevalent method of either raising or lowering the kymograph in its entirety, or on the other hand, of raising or lowering the recording apparatus.

*From Physiological Laboratory, Tufts Medical School.
Received for publication, Feb. 11, 1922.

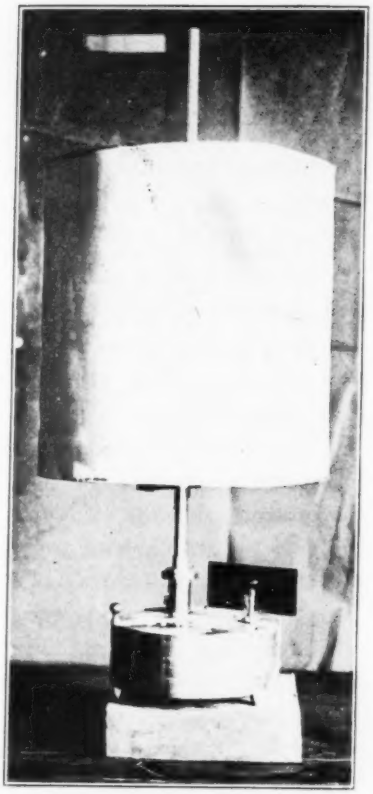

Fig. 1.—Shows drum in position upon the Harvard Motor.

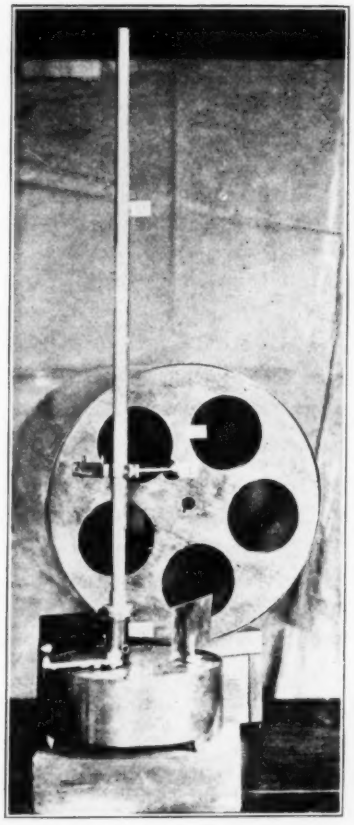

Fig. 2.—Shows the sleeve with the rack and pinion adjustment. 1. Indicates the sleeve. 2. Construction of end of drum. 3. Pinion. 4. Brass bushing.

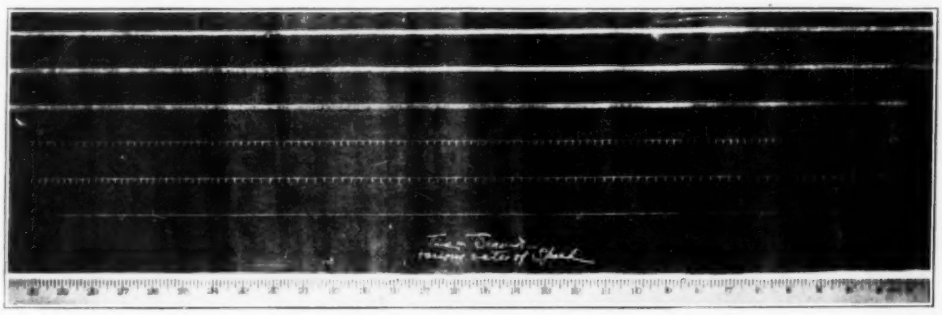

Fig. 3.—Shows photograph of a tracing made to demonstrate the accuracy of this drum. The time being in seconds, records made at various rates of speed.

This adjustment is extremely simple and is brought about by a rack and pinion, the rack being soldered on the sleeve of the drum. This allows instant adjustment in either direction of the drum. The lower end of the sleeve is covered with a brass bushing of sufficient height so that the base of the drum will just clear the governing fan. For some speeds it is desirable to increase the fan surface.

The total cost of this drum was as follows:

| | |
|---|---|
| 1/32" sheet aluminum (90 x 30C) | $1.80 |
| rack | .72 |
| pinion | .15 |
| TOTAL | $2.67 |

Several of these drums were made and have been in use in this laboratory for over a year and have proved most satisfactory.

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## THE PRACTICAL VALUE OF AN ICE-WATER BATH FOR USE IN THE COMPLEMENT-FIXATION TEST FOR SYPHILIS*

By H. Virginia Langworthy and E. Jane Kerley, Albany, N. Y.

THIS paper is merely a preliminary report of some work done at the State Laboratory to compare the degree of complement fixation which takes place at different temperatures: that is, room temperature, ice box temperature and ice-water bath temperature. The object of the work was to show the practical value of an ice-water bath for use in laboratories not equipped with a brine-cooled cold-room.

The range of temperature of an ordinary ice box is between 11° and 16° C. and may approach 18° C. if the ice box is opened too frequently. The brine-cooled refrigerator in use in the State Laboratory maintains a temperature of approximately 3° – 6° C. An ice-water bath, however, can be kept at a temperature as low as 2° C.

Brine-cooled ice box temperature (3° – 6° C.), ordinary ice box temperature (12° C.), ice-water bath temperature (2° C.) and room temperature (20° C.) were used to test a limited number of serums received for the complement-fixation test for syphilis. The majority of these were from cases under treatment for syphilis at Auburn Prison, sent to us by Dr. Heacox to whom we are greatly indebted for his hearty cooperation. The others were either from cases showing clinical evidence of syphilis, or from inmates of prisons or reformatories. All of the specimens tested had given marked or slight fixation in the routine test.

Seventy-one specimens were tested with the plain alcoholic antigen, fifty-eight specimens with the cholesterinized antigen. The effect of the different temperatures of fixation was more evident in the tests with plain alcoholic than with cholesterinized antigen. In the tests with plain alcoholic antigen, but one-third of the specimens tested gave the same fixation at all four temperatures. In the test with the more sensitive, cholesterinized antigen, two-thirds of the specimens tested gave the same fixation at all four temperatures.

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*From the Division of Laboratories and Research, New York State Department of Health, Albany. Augustus Wadsworth, M.D., Director.

Presented at the One Hundred and Sixteenth Annual Meeting of the Medical Society of the State of New York at Albany, April 19, 1922.

Received for publication, June 14, 1922.

TABLE I

COMPARISON OF FIXATION IN THE ICE-WATER BATH AT 2° C. WITH FIXATION IN EACH OF THE FOLLOWING: BRINE-COOLED ICE BOX AT 3° — 6° C., ORDINARY ICE BOX AT 12° C. AND ROOM TEMPERATURE 20° C.

| NUMBER OF SPECIMENS TESTED | WITH PLAIN ALCOHOLIC ANTIGEN 71 | | WITH CHOLESTERINIZED ANTIGEN 58 | |
|---|---|---|---|---|
| | Number | Per cent | Number | Per cent |
| Equal fixation at all temperatures | 23 | 32.39 | 45 | 77.58 |
| Unequal fixation at different temperatures | 48 | 67.60 | 13 | 22.41 |
| Greater fixation at 2° than at 3° – 6° C. | 6 | 8.45 | 3 | 5.17 |
| Greater fixation at 2° than at 12° C. | 29 | 40.84 | 6 | 10.34 |
| Greater fixation at 2° than at 20° C. | 47 | 66.19 | 12 | 20.68 |
| *Less fixation at 2° and 3° – 6° than at 12° or 20° C. | 1 | 1.40 | 1 | 1.72 |

*Differences in these two cases very slight, between negative and plus-minus, and between plus-minus and one plus.

Comparing the fixation in the ice-water bath and brine-cooled ice box, the difference is slight. Six, or less than 10 per cent of the entire number tested with plain alcoholic antigen, and three, or only 5 per cent, of the number tested with cholesterinized antigen gave greater fixation at 2° than at 3° – 6° C. The difference in fixation between the ice-water bath and the ordinary ice box is more marked. Twenty-nine, or 40 per cent, of the number tested with plain alcoholic antigen and six, or 10 per cent, of the number tested with cholesterinized antigen reacted more strongly at 2° than at 12° C. The difference in fixation between ice-water bath and room temperature was much more pronounced. Forty-seven, or 66 per cent, of the specimens tested with plain alcoholic antigen and twelve, or 20 per cent, of the specimens tested with cholesterinized antigen gave greater fixation at 2° than at 20° C.

The degree of variation between the reactions obtained at 2° and 3° – 6° was very slight. That between 2° and 12° was slightly more marked, while that between 2° and 20° was in some cases striking, with such differences as 4+ at 2° and ± at 20°, or + at 2° and negative at 20° C.

The results given were obtained in an improvised ice-water bath, consisting of a small insulated (17 × 11 inches) copper water-bath encased in a wooden packing box lined with heavy paper. The three-inch space between the outside of the bath and the box was filled with flake asbestos. The bath rested on one-inch excelsior pads. By using blocks of ice that nearly filled the lower well of the bath, a temperature of 2° C. and lower was kept for more than four hours. (Nineteen tests with each antigen, or thirty-eight tests altogether could be placed in this bath.)

A household ice box could be easily converted into an ice-water bath. A wooden one lined with zinc having inside measurements 12 by 17 inches and 12 inches deep is sold for thirteen dollars. The outlet pipe of an ice box of this type could be stoppered, the space under the shelf nearly filled with a large piece of ice, and sufficient water added to cover the wire shelf to a depth of 1½ inches. Such a water bath should keep a temperature of 2° or below for four hours or longer, and should prove of great practical value where a brine-cooled cold room is not available.

# AN AUTOMATIC DISTRIBUTING APPARATUS*

By Leon H. Cornwall, M.D., and George Philipp Schmitt, New York City

THE apparatus illustrated in the accompanying photographs is designed to facilitate the performance of work in laboratories where a large number of serologic, immunologic or chemical tests are routinely performed. Its function is to distribute rapidly a measured amount of any required liquid ingredient to a large number of test tubes or other containers.

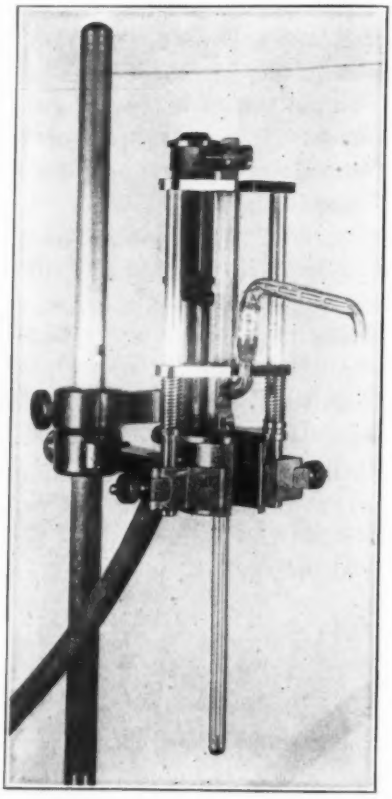

Fig. 1.—Enlarged view of the distributing apparatus illustrating the detailed points of construction.

The apparatus consists of two separate sections, one of glass through which the measured liquid is aspirated and delivered, and the other of metal for the automatic operation of the first.

The glass section consists of tubing of the approximate size and calibre of the ordinary 1 c.c. pipette. There is a vertical portion and a curved horizontal portion. In both the vertical and the horizontal portions there are

*From the Pathologic Laboratories, City Hospital, New York City.
Received for publication, June 5, 1922.

bulbous dilatations, each of which contains a conical glass bead. The lower ends of these bulbous dilatations are conical and ground so that the beads fit tightly into them. By this arrangement the beads function as opposing valves. When one valve is open the other is closed and vice versa. This allows fluid to be aspirated into a syringe that is connected to the upper end of the vertical arm and delivered through the curved horizontal arm. The syringe may be fitted into a ground aperture adapted therefor and removed when desired or it may be a continuation of the vertical tubing.

The operating mechanism is of metal and on the principle of a vertically disposed syringe. The piston, instead of being operated by traction, is elevated by air forced into the barrel from a bulb that is manipulated by the operator. The piston descends by gravity when the pressure on the

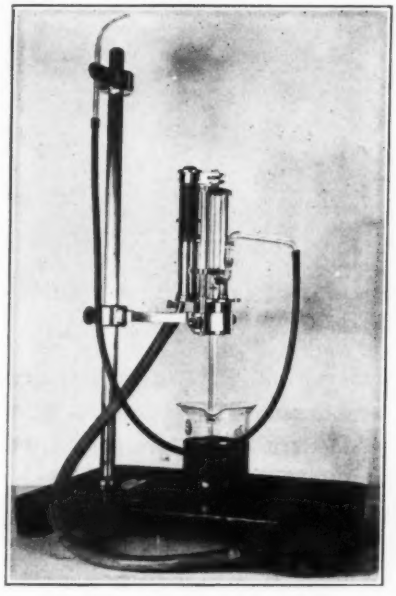

Fig. 2.

Fig. 3.

Fig. 2.—Showing the distributing apparatus attached to a ring stand and arranged for the distribution of antigen for the Wassermann reaction.

Fig. 3.—Showing the distributing apparatus attached to a bottle of colloidal gold reagent.

bulb is released. On one side of the metal piston there is a lug which projects through a longitudinal slit in the barrel. The outside of the barrel is threaded and has a nut which may be screwed up or down. The excursion of the piston is regulated by the adjustment of the movable nut against which the lug on the piston impinges when air is forced in. An arm projects from the upper end of the metal piston and engages the upper end of the glass piston.

The whole apparatus may be attached to a ring stand or clamped to the neck of a bottle or flask. The metal frame to which the glass portion is clamped is light and easily movable. When it is not expedient to utilize the operating mechanism an ordinary syringe or an automatic syringe (see

Jour. Am. Med. Assn., Feb. 18, 1922, p. 506) may be attached to the capillary valve tubing.

When maintenance of sterility is essential the glass parts may be sterilized by boiling and the vertical tube can be inserted through the stopper of a bottle or flask. Contamination may be avoided by flaming the glass tip before use and then sealing it by some simple device such as hot paraffin.

This apparatus will distribute all of the ingredients in serologic work in less than one half of the time ordinarily consumed. It is especially useful for the addition of colloidal gold solution to a large number of tubes. All of the parts are easily detached and assembled. When it is necessary to use clean glassware for each ingredient and simple washing with saline does not accomplish sufficient cleansing the glass section can be removed and a new one inserted in a few seconds. The several parts can be obtained separately and with one operating section any desired number of glass sections may be used.

This apparatus may be obtained from the Will Corporation, Rochester, New York. Trade name "VOL-U-METER."

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## A CONTINUOUS WATER STILL FOR A BACTERIOLOGICAL LABORATORY

By Merlin L. Cooper, Chicago, Ill.*

THIS continuous all glass water still was devised for use in a bacteriological research laboratory. The advantages of the still are that it utilizes but one source of water for both condensing and distilling; maintains a constant water level in the flask of boiling water by automatically feeding the flask with hot water as needed to replace the water evaporated; runs continuously with little attention; and has a capacity of 1 liter of distilled water per hour for each flask connected into the system.

As shown in Fig. 1 the operation of the still is as follows: Fresh water from the water line passes through glass tube *A*, enters condenser *B*, passes out through glass tube *C* and enters beaker *X* through one of its side arms *D*. From this beaker a small amount, as is required, passes out through another side arm *E*, then through bent connecting tube *F*, and enters the second beaker *Y* through one of its side arms *G*. From this beaker (*Y*) the water, as it is needed in the flask, passes out through side arm *H*, bent glass tube *I*, and enters flask *Z* through side arm *K*.

The water from the condenser, which is not needed to maintain a constant water level in beaker *Y* and flask *Z*, passes out of the first beaker (*X*) through its third side arm *N* to the waste drain through glass tube *O*.

Beaker *X* should be just a little higher than beaker *Y*, and beaker *Y* a

*Research Bacteriologist, Morris & Co.
Received for publication, March 20, 1922.

little higher than flask *Z*. Side arms *D* and *G*, supplying the water to their respective beakers, should each be slightly higher than the other side arms.

The water in beaker *Y* is maintained at a steaming temperature by conduction of heat from the boiling water in flask *Z*. In this way this water, as it enters the flask to replace the water evaporated, is already at a high temperature and the boiling of the water in the flask is not interrupted.

In this laboratory a second flask, the same as flask *Z*, was quite often attached to beaker *Y* through side arm *P* and a bent glass tube similar to *I*. This required the use of another gas burner and condenser as the only pieces

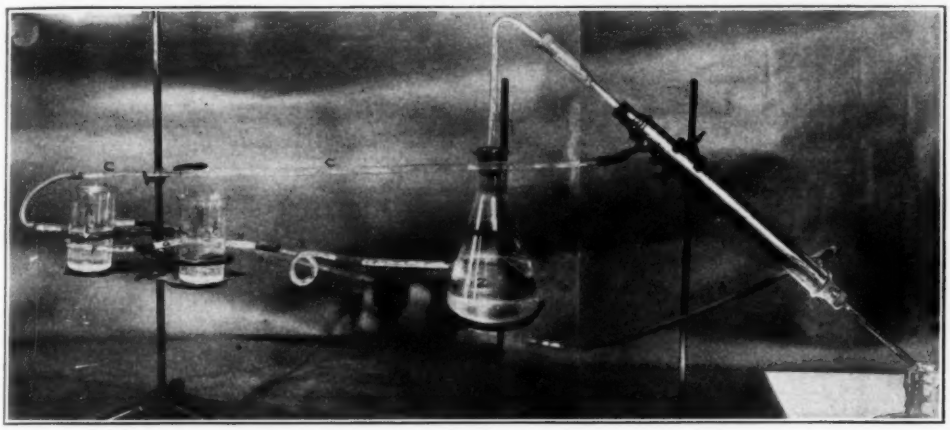

Fig. 1.—A continuous glass water still.

of extra apparatus. The water supply for this flask was derived from the hot water in beaker *Y*, the same as for flask *Z*. This double flask system gave a capacity of 2 liters of distilled water per hour. More flasks could be attached to beaker *Y* by attaching more side arms to this beaker, thus increasing the capacity. The same supply of cold water could be utilized for several condensers by having as many of them attached one with another as is found efficient in maintaining the condensers sufficiently cold to condense all steam.

# A CLASS DEMONSTRATION OF INTESTINAL ACTIVITY*

By A. E. Guenther and Homer C. Lawson, Omaha, Nebr.

OF ALL the ordinary class demonstrations none presents such uncertainty in results as the one dealing with intestinal movements. The usual "immersion method" first used by van Braam Houckgeest[1] in 1872, in which the abdomen of an animal is opened under a suitable saline solution seldom yields more than an insignificant activity. As a demonstration method to students the immersion method has the great disadvantage that comparatively few observers can gather about the tank to view the movements, if present. More than a year ago, the authors, under the necessity of demonstrating the effects of various drugs upon intestinal activity, conceived the plan of preparing a light adjustable board, provided with a central opening through which the intestines of an operated rabbit might hang. The board serves as a support to the body of the animal and can be lowered until the pendent intestines enter a saline solution in a jar placed beneath the board. The intestines buoyed up by the solution can be viewed by a class of ordinary size from any point within the confines of an ordinary class room. The demonstration is improved if a flat anatomical preparation jar of clear glass is selected and the intestines are illuminated by the use of a properly shaded incandescent light.

The method gave disappointment at times when the intestines failed to hang down into the solution owing to the buoyancy imparted to them by contained gas. It served, however, as the point of departure of a series of trials which eventuated, finally, in the method to be described and which gives so striking a demonstration of intestinal activity that all having witnessed it are impressed. The method to be described requires the cooperation of two persons in the preparation for a demonstration.

## METHOD

The animal is anesthetized preferably with a permanent anesthetic. Urethane is satisfactory for rabbits. After removing the abdominal hair an incision is made in the median line approximately four and one-half inches in length, beginning one inch posterior to the tip of the xiphoid and involving only the skin. The skin is then separated from the underlying abdominal muscle by separating the fascial planes, avoiding hemorrhage. The dissection should extend about one inch anteriorly and posteriorly beyond each end of the incision and laterally from one and one-half to two inches in both directions. An eight inch ligature is now drawn through the adjacent right and left edges of the skin incision about one and one-half inches from its

*From the Pharmacological Laboratory of the University of Nebraska College of Medicine, Omaha. Received for publication, July 5, 1922.

anterior end and allowed to lie in place. (Fig. 1, B, lig.) An incision in the linea alba, opening into the abdominal cavity, should be made somewhat anterior to the center of the skin incision and not exceeding three-quarters of an inch in length. With caution a pair of forceps may now be introduced through the slit towards the animal's left and a loop of the small intestine drawn upwardly and out through the opening. With the fingers, previously moistened, and, exercising care, ten to twelve inches of the intestine may be gently drawn through the opening and allowed to rest on the exterior of the abdominal wall. It is possible, as a precaution, if obstruction of the intestinal lumen is feared, to introduce into the slit and fasten in place a hard rubber ring two centimeters in diameter, preliminary to drawing out the intestines. This, however, was not found essential to the success of the demonstration.

If the intestines are found to be empty and flat, it is desirable, at this

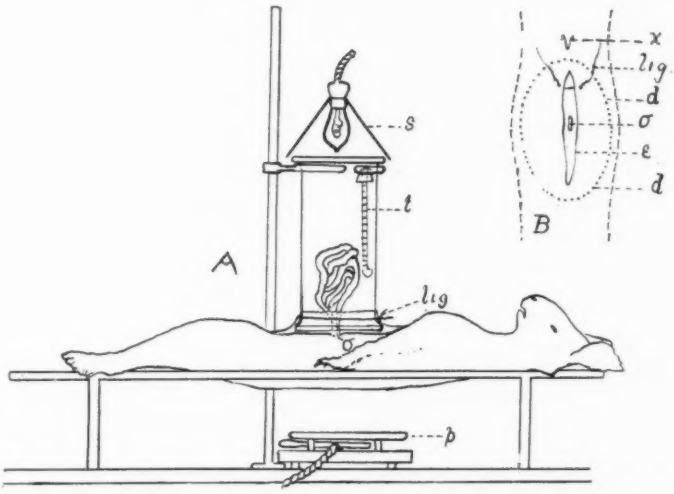


Fig. 1.—(*A*) Sectional diagram of apparatus to demonstrate intestinal activity. *t*, thermometer; *s*, shaded light; *o*, opening into abdomen; *p*, electric warming plate.

(*B*) Diagram of operative field. *x*, xiphoid cartilage; *lig.*, ligature; *o*, opening into abdomen; *i*, incision in skin; *d*, area of separation of skin from underlying musculature; *e*, edge of incision into skin.

time, to introduce at various points along the loops a small quantity of air by means of a hypodermic needle. This will render the loops of the intestine buoyant, when later, they are immersed in saline solution. A cylindrical museum jar, three and one-half by seven inches and provided with flanges at base and top, is almost filled with Tyrode's solution which has previously been brought to the temperature of the animal's body. A short thermometer, inserted into a cork to serve as a float, is placed in an inverted position in the jar.

The assistant now grasps the forelegs and ears of the rabbit with one hand and the hind legs with the other and places the animal over the jar so that the intestines hang into the solution. While the assistant thus holds the animal in place, the operator draws the loosened skin over the flange of the jar and stretches it in place by means of the ligature mentioned above.

(Fig. 1, A, B, lig.) It will now be necessary to remove the layer of air separating the abdominal wall from the solution in the jar. To do this the operator inserts a rubber tube between the anterior edge of the incision and the jar and by means of a funnel at the other end of the tube introduces warmed Tyrode's solution which displaces the air. By suitable manipulation all of the air may be expelled easily.

By proper cooperation between the experimenter and the assistant the jar and the animal are rapidly inverted. This maneuver is made possible by the experimenter placing one hand on the back of the animal while the other hand grasps the jar and holds it in constant contact with the body of the animal while the inversion is being made. The inverted jar is now held by a suitable ring support so that its weight and that of the contained column of liquid do not bear down on the body of the animal. The buoyant intestinal loops float up into the jar and can be viewed by a large circle of students. The effect is greatly enhanced by slightly darkening the room and illuminating the intestines from above by means of an incandescent bulb as shown in the illustration. (Fig. 1, A.)

It should be borne in mind that the intestines are not under hydrostatic pressure but under normal atmospheric pressure. Neither need the junction between the skin and the jar be water tight. The relations between jar and rabbit are precisely those existing between an inverted tumbler of water and the sheet of paper which, by atmospheric pressure, serves to keep the water from falling out.

## EXPERIMENTAL RESULTS

After placing the intestines in a jar, a variable period of time ensues during which the intestines are practically inactive. The period of inactivity, in rabbits, varies from one-half to two hours. In the interval between the second to the fifth hours the activity reaches a maximum but periods of marked activity alternate with periods of relative quiescence. Rhythmical segmentation with alternate flushing and blanching of the segments may be observed in some instances. Peristaltic waves, more or less pronounced, are common. The most frequent and striking movement, however, is the swaying of the loops from side to side, usually complicated by peristalsis. The swaying or pendular movement is, undoubtedly, more pronounced than in the closed abdominal cavity owing to the free manner in which the loops are poised in the solution. Upon these intrinsic movements of the intestine are superimposed the rhythmical thrusts of the pulse and respiration. Occasionally borborygmi are clearly audible at a distance of three to five feet from the preparation. A surprising and unusual spectacle is the drawing of the intestinal wall over the stationary fluid contents within the lumen. That the contents are stationary is established by the presence within the fluid of suspended darker masses plainly visible through the illuminated translucent intestinal walls. Taken all together, the movements are puzzling and complicated but in the course of an hour it is usually possible to witness the typical activities described by Starling and Bayliss.[2]

Although the transportation of intestinal contents (with the exception of the to and fro movements) is not clearly appreciable, it obviously must take place since there is an accumulation of material at the distal end of that portion of the intestines lying outside of the abdominal cavity. Peyer's patches clearly visible in the intestinal wall do not take an active part in the contraction of the walls of the gut, and, consequently, stand out from the general contour of the wall as well defined nodules. The flattening of the intestines when empty is worthy of mention.[3] In one experiment on a rabbit carotid blood-pressure tracings were made during the course of a demonstration. The results are shown in Table I.

TABLE I

SHOWING EFFECT OF DEMONSTRATION ON BLOOD PRESSURE IN A RABBIT

| TIME | BLOOD PRESSURE | REMARKS |
|---|---|---|
| 2:00 p. m. | 72 mm. Hg. | Four hours after urethane administration |
| 2:30 p. m. | 60 mm. Hg. | Two. mins. after operation and intestines in jar |
| 3:30 p. m. | 66 mm. Hg. | Intestines quite active |
| 8:30 p. m. | 66 mm. Hg. | Intestines slightly active |

During ordinary summer days the temperature of the solution in the jar as indicated by the enclosed thermometer remains practically constant. The maintenance of the temperature is mainly due to conduction from the body of the animal. On cold days this source of heat may be reenforced by the heat from the incandescent lamp above and by the cautious use of an electric hot plate under the animal board.

This method of demonstrating intestinal movements is of particular service in pharmacology. During a period of lively intestinal activity in a rabbit the hypodermic administration of thirty milligrams of morphine sulphate produced complete cessation of all intrinsic movement within five minutes after a preliminary augmentation. Nicotin, pilocarpin and atropin promptly produced the anticipated results, those produced by pilocarpin being especially striking.

REFERENCES

[1]Van Braam Houckgeest: Pflueger's Arch., 1872, vi, 266.
[2]Starling and Bayliss: Jour. Physiol., 1899, xxiv, 99; ibid., 1901, xxvi, 125.
[3]Engelmann: Pflueger's Arch., 1871, iv, 37.

# BARBITAL AS AN ANESTHETIC FOR DOGS*

By Arthur L. Tatum, M.D., and Eloise Parsons, B.S., Chicago, Ill.

IN EXPERIMENTS on dogs lasting throughout a number of hours, investigators have been considerably handicapped on account of deleterious side actions produced by the ordinary anesthetics such as ether or chloretone. Ether itself produces a number of complicating actions and in addition it is impossible to maintain with ether a constant depth of anesthesia. Chloretone is notorious for the prolonged anesthesia obtainable but with the serious objectionable features of progressively lowering blood pressure.

In routine class-room study of the hypnotic series of drugs in our laboratory, it was observed that with a sufficient dosage of barbital the effects of depression to the state of complete surgical anesthesia persisted beyond twenty-four hours. After twenty-four hours the animals were used for acute experiments. The blood pressure was entirely within the normal values of anesthetized animals, while the vasomotor responses were equally as good as those observed during ordinary light ether anesthesia. Pancreatic secretion is as good as that under ether while gastric secretion after secretin and renal secretion from diuretics were far superior to that observed under ether. "Normal" urine secretion during barbital anesthesia appears to be as good as that produced by diuretics under ether anesthesia even in most favorable experiments.

While the pharmacology of "veronal" has been intensively studied by Jacoby and colleagues, we are unaware of the use of barbital in place of other anesthetics. We believe it to be as well adapted for prolonged anesthesias in dogs as is urethane for rabbits. It has a very great advantage over chloretone insofar as barbital does not appear in our experience to produce any fall in blood pressure which is the serious objection to the use of chloretone.

While we make no particular claims for originality in the use of barbital as an anesthetic for dogs, we are not aware of its having been so employed. We feel that the calling attention to this use of barbital may be of considerable service to investigators in carrying out prolonged experiments.

The technic we have employed is as follows: Barbital sufficient to make 0.25 g. per kilo. body weight is dissolved in dilute sodium carbonate solution. This solution administered by stomach tube produces surgical anesthesia in from one half to one hour and lasts for at least eight hours. With a dosage of 0.35 g. per kilo., anesthesia lasts for at least twenty-four hours. By avoiding an excess of sodium carbonate over that required for solution of the barbital, vomiting is not apt to occur. The solid form of barbital may be given equally well in suspension in any suitable vehicle such as acacia or starch solution.

*From the Laboratory of Physiological Chemistry and Pharmacology of the University of Chicago.

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## EDITORIALS

### *Variations of Virulence in Tubercle Bacilli*

IN A RECENT address Dr. E. R. Baldwin[1] spoke of attenuation in old cultures of tubercle bacilli, and emphasized our unfortunate lack of any standard procedure whereby to test the relative virulence of different strains.

Variation is one of the most fundamental known laws of biology. All organisms exhibit it to some degree in all their measurable characteristics. Variation among pathogenic microorganisms of the same species has long been a favorite theme of Dr. Theobald Smith's, and recent communications by him,[2] and by Dochez,[3] Topley,[4] and Flexner and Amoss,[5] illustrate our growing knowledge and interest in this fascinating field. The familiar variations in morphology among tubercle bacilli recovered from any large series of cases are an example of the general law.

From this principle alone one could predict with confidence variations of virulence in tubercle bacilli; and conspicuous examples are known, in particular the Saranac Lake R1 strain, to which Dr. Baldwin referred. This

strain originally appeared to be of standard virulence for the guinea pig, but after two or three years of cultivation its pathogenic power decreased greatly and rather rapidly. Now for nearly thirty years guinea pig inoculations with emulsions of this organism have produced with great constancy a disease which is definitely tuberculous, but very benign, and from which the animals recover, with increased resistance to subsequent inoculation with virulent strains.[6, 7] On the other hand, different strains which have been cultivated at Saranac Lake under the same conditions for seventeen and twenty years have not as yet changed appreciably in virulence.[1]

As Krause says, many workers with tubercle bacilli have noticed attenuation under prolonged cultivation,[6] and it has been noted in other circumstances as well. Perez[8] and Manfredi and Frisco,[9] long ago reported the low pathogenicity of bacilli recovered from the lymph nodes of animals inoculated on the surface of the skin and mucous membranes. In our laboratory we have produced relatively mild and chronic disease by inoculating guinea pigs with caseous guinea pig lymph nodes which had been incubated for a few days[10, 11] (and subsequent unpublished work). Cobbett[12] mentions, and many have noticed, the comparatively benign disease caused by inoculation with drying cultures which are not yet entirely dead; and Calmette[13] has reported that marked and rapid reduction of virulence can be effected constantly by the use of bile media.

In some of these cases it is doubtful whether we are dealing with true attenuation or merely with diminished numbers of living bacilli; but in the case of the R1 strain, and of Calmette's bile cultures, there is undoubtedly genuine reduction of virulence, and some of our recent results may have a similar explanation.

Such examples of variation under artificial conditions are of interest, and may be of practical importance in making protective vaccination possible. But it would better help our understanding of the parasite in its relation to the type of disease produced if we could detect variations of virulence in strains freshly isolated from the natural host. On this subject we have remarkably little information, and, as Dr. Baldwin says, not even a reliable method.

The state of our knowledge is admirably set forth in Cobbett's "Causes of Tuberculosis." Briefly, we have the three known races of tubercle bacilli, human, bovine, and avian. On the basis of growth characteristics on artificial media, and of disease produced in guinea pigs, rabbits, cattle, and birds by inoculation with large doses most strains of tubercle bacilli can be quite definitely classified in one of these races, which appear to have a differentiation about as clear as that of Caucasian, Mongolian, and Negro. There is, however, a minority of strains, for example those isolated from lupus, which do not conform exactly to any of these types, and over these strains there is heated controversy, as to whether they are eccentric members of specific races, or transition forms between two races.

Instead of solving this difficulty, study of the data so far available raises new perplexities. Granting that the human type—or the bovine type, which

lends itself best to study—is a distinct race, or subspecies, something fixed, as far as the word is admissible in speaking of living organisms, what about the variations within such a class? If we take only those strains which in the doses commonly employed cause progressive disease in rabbits and cattle, and do not grow vigorously on artificial media—the typical bovine bacilli—how much do they vary in minimum lethal doses for animals of the same breed, age, and weight?

But little work has been done on this problem, which obviously calls for large resources and experiments *en masse*. Two methods suggest themselves: First, the old process of diluting emulsions, the technic of which is described by Calmette;[13] second, the method of Barber, which consists of picking up known counted numbers of bacilli with fine capillary pipettes under the high power of the microscope.[14] The objections to the first method were picturesquely phrased by Cornet in another connection, when he said that it was as inexact to speak of diluting tubercle bacilli in a test tube as to speak of diluting crocodiles in a river.[15] The objections to the second method have been stated by Krause.[16] It is clearly true, as Krause says, that one cannot be certain that all the bacilli picked up are alive, or that they all enter the tissues of the inoculated animal, or that the count is not falsified by several closely cohering bacilli being counted as one. Nevertheless, having made a great deal of use of the Barber technic, we feel sure that for the purpose under discussion it involves less chances of error than the other, or any other method.

In the course of our studies on immunity production by inoculation with living organisms, beginning with one bacillus, in which the Barber method was employed,[17-23] we made a few observations on variation of virulence in cultures, as shown by the minimum number of bacilli which caused visible lesions.[18, 19, 23] The problem should, however, be worked out with many freshly isolated strains from various sources, eliminating, as far as possible, variations developing under the artificial conditions of cultivation.

In such an investigation, any conceivable method involves chances of inaccuracy, which can be minimized by standardizing the technic as rigidly as possible and employing large numbers of experimental animals and controls. When the subject is approached in this way, somewhat as the British Royal Commission approached the problem of types, we shall get some valuable information on spontaneous variations in virulence among tubercle bacilli of the same race. We can then ascertain whether virulence is enhanced by contact with new host material, as seems to be the case with some epidemic diseases, and shall be in a better position to gauge the variations in resistance of different human races and groups.

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—*G. B. W. (C. T. R.)*

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## *Treatment of Hookworm Infection with Carbon Tetrachlorid*

HALL[1] advocates the use of carbon tetrachlorid in the treatment of hookworm infection. He claims that one dose of .3 c.c. for every kilogram of body weight is sufficient to remove almost all worms. He tested this agent on monkeys, giving as much as five times the dose mentioned above, without producing unfavorable symptoms. He himself took in the morning 3 c.c. of carbon tetrachlorid and did his usual day's work without any inconvenience. The drugs which have been most in favor in the treatment of this disease are thymol, beta-naphthol, and Chenopodium. All of these need to be administered in considerable doses and with caution, since serious results have been known to follow. Carbon tetrachlorid can be prepared cheaply and obtained quite pure. It is a colorless liquid, with a sweetish pungent taste. It is soluble in alcohol and ether and itself dissolves fatty substances. The only medical use to which it has been employed heretofore is as an anesthetic and as an inhalation in hay fever. At one time it was used by the barber in dry shampoo, but fell into disuse because a woman died suddenly after its application. There was some question as to the cause of death.

Nichols and Hampton[2] have taken up this subject and tried it out both upon healthy people and upon those harboring the hookworm. A murderer sentenced to death volunteered to take 6 c.c. This was given one hour after the midday meal and some hours later he passed four ascaris worms. Thirteen days later this dose was repeated in the early morning before breakfast. The patient stated that during the forenoon he felt a little giddy and sleepy, but this soon passed. He was executed a week after he had received the second dose and a postmortem done one hour after death showed no worms in the intestines. Besides, the autopsy showed no lesions which could be attributed to the dose administered. In an agricultural college in Ceylon carbon tetrachlorid was tried out on the students. No purgative was given

[1]Jour. Am. Med. Assn., November 19, 1921.
[2]British Med. Jour., 1922, i, 8.

on the preceding night, all received a dose of 3 c.c. of the drug before breakfast, and no purgative was given after the drug had been administered. The students were instructed to carry on their usual mode of life and no restrictions were placed upon their work, play, or diet. No inconvenience from the drug was recognized, and an average of thirty-six hookworms for each student was recovered from the stools. Next the drug was tried on sixty-four additional students, with like results.

Nichols and Hampton conclude: (1) Carbon tetrachlorid is an efficient anthelmintic; (2) that the drug may be administered safely in doses of 10 to 20 minims to children of three or four years of age; (3) that it aids in the expulsion of Ascaris lumbricoides if it is followed by a purgative, but in this respect it is not as effective as Chenopodium; (4) that the drug does not seriously deteriorate on keeping; (5) that it is preferable to Chenopodium for the following reasons: (a) there is no objection to its taste; (b) it is not necessary to precede or follow its administration by a purge; (c) it is more efficient than Chenopodium and is not so distressing; (d) it is much cheaper than any other drug used for this purpose; (e) it can be prepared in a high degree of purity and only chemically pure preparations should be used; (f) the patient is not interrupted in his vocation.

—*V. C. V.*

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## *The Excretion of Potassium Iodid as a Test of Renal Function*

LINDER,[1] acknowledging that it is unwise to unduly multiply the tests of renal function, thinks that if a new agent proves to be reliable and gives additional information of use in diagnosis, prognosis, or in treatment, it will justify the experimentation. He thinks from his experiments that the iodid test is of great value in prognosis. He has classified his cases according to their iodid output. The patients who excreted less than fifteen per cent of that administered within a given time had the clinical signs of severe renal damage. They showed no tendency to improve; in fact, most of them steadily deteriorated and died in a few months. In the group from which he extracted from fifteen to twenty-five per cent of the iodid, there were two types of cases: (a) Those in whom the power of excretion did not recover and whose subsequent progress was unfavorable, and (b) those in whom the iodid excretion did recover and whose progress was good. All of the last were cases of acute nephritis. Of those who excreted from twenty-five to thirty-five per cent, two recovered the power of iodid elimination and of these one progressed well and one moderately well considering the unfavorable environment to which she returned. The one who failed to improve his iodid excretion showed practically no amelioration of symptoms even while under hospital treatment. The improvement of the iodid output is a valuable sign of recovery. The progress of the patients who eliminated more than thirty-five per cent was good, so far as renal conditions are concerned, but

[1]Quart. Jour. Med., April, 1922.

unimpaired power of iodid elimination is of less significance than its impairment.

Linder's conclusions are: (1) The iodid test is a reliable indication of nephritis when the excretion falls below thirty-five per cent; (2) in certain cases of nephritis it is the only test of function which shows renal damage; (3) it is a good sign when the excretion remains unimpaired throughout; a fair prognosis when it recovers after being impaired and a bad prognosis and of advanced structural damage when it remains indefinitely in the region of twenty-five per cent. A reduction of the output to fifteen per cent or less is a sign of advanced progressive disease and often foretells death. (4) In certain cases it may be an aid to treatment, indicating a less advanced stage of recovery than can be recognized by clinical signs and other function tests; (5) rarely some iodism is noticed, but in no case was there evidence of a deleterious effect on the kidney.

—*V. C. V.*

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## *The Relapsing-fever Spirochete of Panama*

ST. JOHN and Bates[1] demonstrated about a year ago that the relapsing fever in Panama is transmitted by a tick, Ornithodorus talaje. They inoculated successfully through the tick, white rats, mice and monkeys. In the paper now under review they have compared the Panama spirochete with those of Obermeier, Novy, Koch, and Dutton. Their conclusions are that the spirochete of relapsing fever in Panama is a distinct species, variety, or strain as compared with the others. Relapsing fever in Panama is probably due to one species, variety, or strain of the relapsing fever spirochete for the present called the Panama spirochete.

—*V. C. V.*

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## *Typhus Fever in Boston*

SHATTUCK[2] has studied the records of the Boston City Hospital for the past ten years and arrives at the conclusion that the typhus problem in that City has not been significant from the diagnostic standpoint. However, he thinks that the potential epidemiologic significance of typhus, especially of atypical cases, requires that all suspected cases be treated with the greatest care in order to arrive at a correct diagnosis. Under possible cases, he includes a considerable group having eruptions or temperature curves suggestive of typhus. In this group there are two classes: First, those undiagnosed, and second, those in which diagnosis is not supported by exact facts. Careful investigation of such cases would perhaps show that typhus fever is in reality more common in Boston than it appears to have been in recent years. The Widal reaction is commonly present in typhus fever. Therefore,

[1]Am. Jour. Trop. Med., 1922, ii, 251.
[2]Am. Jour. Tropical Med., 1922, ii, 225.

it is not *per se* an obstacle to the diagnosis of this disease. The proteus reaction of Wilson, Weil and Felix is not infallible, but is a valuable aid. In order not to be misled by this reaction it is necessary that it should be adequately controlled. From time to time the test should be checked with known typhus serum. In the interpretation of the results of this test all known facts regarding it should be borne in mind and clinical evidence should be considered. Shattuck thinks that the surest means of diagnosing typhus fever during life is by histologic examination of excised bits of skin. Pathologic changes found in the guinea pig after inoculation with material containing the virus are believed to be constant, but are sometimes difficult to demonstrate. Inoculation of guinea pigs may be used as an aid in diagnosis. Of great importance for diagnosis is the almost constant absence of leucopenia in typhus and its nearly constant presence in typhoid. There is an increase in number of cells in the cerebrospinal fluid, but this may be slight or marked. Failure to recognize this may lead to erroneous interpretations. Wiener's color reaction is easily made and warrants further trial.

—*V. C. V.*

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## *A Death Caused by Ascarides*

CALDWELL[1] reports that in the village of El Pilon in the Province of Panama in 1921 a baby girl, twenty-two months of age, died under the following conditions: She had been given at 6 A.M. one-half minim of the oil of Chenopodium and this was followed two hours later by Epsom salt. There being no movement of the bowels and no disagreeable symptoms appearing, after two hours more a saline enema was employed. This was followed by evacuation of innumerable ascarides. About 5 P.M. the child became very weak and ascarides in bunches came from her mouth and nose, collected in her throat, blocked the larynx, and caused death from failure of respiration. Ten hours after death, ascarides were still coming from her mouth.

This quite equals the stories found in some of the older reports of the prevalence of this parasite in the Tropics.

—*V. C. V.*

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## *War Nephritis*

DYKE[2] has made a study of the remote effects of this disease. It will be remembered that during the War the men in the trenches were inclined to develop evidences of nephritis, characterized by albuminuria, hematuria, and edema. Some guesses were made at that time as to what the ultimate result would be. Dyke has taken one hundred typical cases of war nephritis, and with the aid of the records of the Pension Department he has followed

[1]Am. Jour. Trop. Med., 1922, ii, 213.
[2]Quart. Jour. Med., April, 1922.

up their histories to the middle of 1921,—a period of about four and one-half years from the onset of the disease. Of the one hundred cases it was found that three had died; one aged thirty-seven; one, twenty-six; one, twenty-nine. One of these died of scarlet fever, and it is a question whether the nephritis had anything to do with the fatality of the scarlet fever. The cause of death in the remaining two cases was given as nephritis, and it seems that in these instances the war nephritis became chronic and led to a fatal termination in eighteen and twenty-seven months respectively. Of the ninety-seven remaining cases, sixty-five were eventually returned to duty, but of these, two had recurrences while serving in France and finally became pensioners on account of this condition. The remaining thirty-two were discharged as unfit.

Dyke's conclusions are stated as follows: (1) Seventy per cent of cases of war nephritis at all ages terminated in recovery before the lapse of one year; (2) the proportion of cases so terminating is slightly higher under than above the age of thirty-five; (3) cases not fully restored to health by the end of twelve months became chronic; (4) the occurrence of symptoms of uremia at the outset has no unfavorable significance as regards the later prognosis; (5) in cases becoming chronic there is a tendency for fatal issue to be determined by circulatory or respiratory conditions; (6) the proportion of deaths from pulmonary tuberculosis among patients in the chronic stage is high.

Dyke calls attention to the fact that early in the War, Osler cautioned against giving too favorable a prognosis. Abercrombie has reported on the after-histories of 171 cases for periods from twenty-four to thirty-two months from onset. His observed mortality at all ages for that period is three per cent; the same as that observed by Dyke.

—*V. C. V.*